ORAL KERATOSES:
A CLINICAL, PATHOLOGICAL AND IMMUNOLOGICAL STUDY

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A Thesis submitted for
the Degree of
Doctor of Philosophy

Faculty of Medicine
University of Edinburgh

The Research was conducted in
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Submitted
1990

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ACKNOWLEDGEMENTS

I am most grateful for the help I have received in completing this Thesis and I am indebted to Dr David Wray, Senior Lecturer in the Department of Oral Medicine and Oral Pathology at Edinburgh Dental Hospital and School, for his supervision and encouragement during the study.

I would also like to thank Professor John Southam of the Department of Oral Medicine and Oral Pathology and Dean of Dental Studies, for his valuable advice and his help with the histopathological assessment of oral keratoses. Within the same department I am indebted to the Reader, Dr Jeffrey Sofaer, for his assistance with computer analysis and initial direction with the computerisation of data.

Evaluation of serum immunoglobulins was made possible with help from the Consultant Dr Peng Lee Yapp and Mr George McNeil, Chief Medical Laboratory Technician within the Edinburgh Regional Blood Transfusion Service at the Royal Infirmary. Furthermore, the assessment of the lymphocyte transformation was made possible using a Beta counter in the Department of Bacteriology and Virology in the University of Edinburgh. I am also grateful for help obtained from Dr Andrew Krajewski, Senior Lecturer in the Department of Pathology, University of Edinburgh who advised on the selection of suitable monoclonal antibodies for immunocytochemical staining of the oral keratosis sections. Furthermore, technical advice obtained within the same department, was invaluable in establishing the monoclonal staining in our laboratory. I am also indebted to the Lothian Health Board who

funded £1500 for the purchase of reagents for the immunocytochemical work.

I am also most grateful for the food intolerance and sugar absorption investigations completed with the Professor Anne Ferguson, in the department of Gastroenterology at the Western General Hospital.

Mr John Mitchell, Chief MLSO within the Department of Oral Medicine and Oral Pathology, has been a constant source of encouragement and help with the multiplicity of laboratory requirements during the study. In addition, Mr Ian Morrison from the Department of Conservative Dentistry, helped with initial computerisation of the ELISA assays. Also, the replacement of the metal crowns with ceramic crowns for the patient illustrated in Figures 16 and 17, was made possible with the help of Mr Kenneth MacKarron, dental instructor in Conservative Dentistry.

I wish also to thank Mrs Dawn McKinnon for her help with the routine histopathology and immunocytochemical slides. The data manipulation using the statistical system SPSSX was greatly aided by Ms Barrie Wohlgemuth, the Computer Officer in the Department of Preventive Dentistry, without whose help the analyses would have been almost impossible. I would like to thank the Hospital Records Staff who helped with the constant retrieval of patient notes for the study.

The librarians in the Erskine Medical Library and the library of the Royal College of Physicians provided an invaluable service with the retrieval of papers and journals. I am also most grateful for Miss

Jean Adams' help with the historical aspects of the literature.

Finally, for the support upon which this thesis was more than dependent, I wish to record my sincerest thanks to my wife, Margaret. I would also like to express my gratitude for her dedicated help and for the typing of the scripts as well as controlling my young daughter, Emma, during the completion of the Thesis.

DECLARATION

I declare that this Thesis is entirely my own work.

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ABSTRACT

The diagnosis of a patient presenting with an oral keratosis is the first stage in the management of the patient. Diagnosis is based on a combination of patient history, clinical examination and laboratory investigations. The aim of this thesis was to identify factors which may be helpful in the diagnosis of oral keratoses. It is based on a prospective study of 159 patients being examined haematologically, immunologically and histopathologically. Two main groups of keratoses were identified by the presence or absence of an associated inflammatory cell infiltrate. Infiltrated keratoses comprised patients diagnosed as lichen planus, leukoplakia, discoid lupus erythematosus, candidal leukoplakia and squamous cell carcinoma. Non infiltrated keratoses also comprised patients diagnosed as leukoplakia but also comprised frictional and smoking keratoses and hereditary keratoses. A third category, intermediate infiltrated leukoplakia was distinguished by changeable clinical and histopathological appearances during the study period.

Individual patient diagnosis followed clinical examination and immunological and histopathological investigations. Cross-tabulation of the diagnostic categories with the clinical presentations of the keratoses showed that the clinical appearances may be common to different diagnostic categories. Non infiltrated leukoplakia was seen frequently to involve the floor of mouth. All categories of keratoses had a high proportion of patients currently taking medication: non steroid anti-inflammatory drugs were more frequently taken by patients with erosive lichen planus (29%) than those with non erosive lichen

planus (3%). Abnormal intestinal sugar permeability was detected in 65% and 60% of lichen planus and infiltrated leukoplakia patients respectively but not in any patients with non infiltrated keratoses. Haematological deficiencies were detected in 23 patients (14.4%) of the whole study group and were related particularily to lichen planus and non infiltrated leukoplakia, 22.5% and 19.2% respectively. Tobacco habits were positively associated with categories of leukoplakia and squamous cell carcinoma. Patients with lichen planus had significantly lower tobacco and alcohol use than infiltrated ,non infiltrated leukoplakia and the squamous cell carcinoma categories.

Histopathological assessment of the oral keratoses did not identify any singular diagnostic characteristic. Epithelial dysplasia was seen in lichen planus, leukoplakia and squamous cell carcinoma. Inflammatory infiltrate phenotypes were identifiied using the immunoperoxidase system and quantitative differences existed between the diagnostic categories. The T4/T8 ratio was highest for the frictional and smoking keratoses and lowest for intermediate infiltrated leukoplakia. The intermediate infiltrated leukoplakia category also showed significantly lower serum immunoglobulins (IgG, IgA and IgM) than other diagnostic categories. No evidence of macrophage induced suppression for lymphocyte transformation of peripheral blood or lesional lymphocytes was found. Co-culture experiments suggest lesional cells exert suppressor effects for squamous cell carcinoma and not for the categories of leukoplakia.

Glossary of Abbreviations

AET	2-amino ethylisothiouronium bromide hydrobromide
ANB	Antinuclear Antibodies
B-Cell	Bursa Equivalent Cell
CL	Candidal Leukoplakia
ConA	Concavalin A
Cpm	Counts per minute
DAB	3,3 Diaminobenzidine Tetrahydrochloride
DLE	Discoid Lupus Erythematosus
DPX	Distrene Plasticizer Xylene
ELISA	Enzyme-linked Immunosorbent Assay
EMAS	Edinburgh Multi Access System (Computer)
ESR	Erythrocyte Sedimentation Rate
FCS	Foetal Calf Serum
FSK	Frictional and Smoking Keratoses
HBSS	Hanks Balanced Salt Solution
HK	Hereditary Keratoses
IDA	Indian Dental Attenders
IK	Infiltrated Keratoses
ILK	Infiltrated Leukoplakia
INT	Intermediate Infiltrated Leukoplakia
LP	Lichen Planus
NCS	Newborn Calf Serum
NIK	Non Infiltrated Keratoses
NILK	Non Infiltrated Leukoplakia
PAS	Periodic Acid Schiff
PEG	Polyethylene glycol

PHA	Phytohaemagglutin
PWM	Poke Weed Mitogen
RPM	Revolutions Per Minute
SAPU	Scottish Antibody Production Unit
SCC	Squamous Cell Carcinoma
SPSSX	Statistical Programme for Social Sciences X.
STAI	State Trait Anxiety Inventory
T-Cell	Thymus-Dependent Cell
TBJ	Tris Buffered Saline
WHO.ICD.DA.	World Health Organisation International Classification of Disease (Dental Application)

Abbreviations Developed for Evaluation of Tobacco and Alcohol Habits

Cigday	Mean cigarettes smoked per day
Cigyrs	Mean years of cigarette smoking
Cigall	Mean value for total cigarettes smoked
Gramall	Mean value for total grams of pipe tobacco smoked
Garall	Mean value for total number of cigars smoked
Beeryrs	Mean years of beer consumption
Nipyrs	Mean years of spirit consumption
Wineyrs	Mean years of wine consumption
Yrsalk	Mean years of alcohol use
Unwine	Mean units of wine consumed per week
Total	Mean total combining alcohol and tobacco consumption for the specified population

CHAPTER 1

REVIEW OF THE LITERATURE PERTAINING TO ORAL KERATOSES

EARLY HISTORICAL REVIEW

White appearing lesions of the oral mucosa are not unusual and obtain their appearance from the scattering of light through an altered surface. Such changes may be the result of a thickened layer of keratin or altered hydration of the keratin, and conditions presenting in this way are generally regarded as oral keratoses.

Hipocrates (460-370 B.C.) used the term "aphthai" to describe infantile thrush which presents as a white patch (1). Acute pseudomembranous candidiasis (thrush) is characterised clinically by white lesions of the oral mucosa which can be scraped off to leave a raw, bleeding surface and is associated with altered keratinisation (2,3).

The first description of white patches on the oral mucosa in recent times is attributed to Sir James Paget (1851) who described a smoker's patch of the tongue as leukoma (4), a term adopted later by Butlin (1885) in his monograph "Diseases of the Tongue" (5). Morris (1874) later pronounced Hulke's report to the Royal Medical and Chirurgical Society (1864) to be the first description of "ichthyosis glossae", white patches of the tongue or cheek (6). It was then that the association between "ichthyosis" and "epithelioma" was first raised by

Sir James Paget (1864), who was to be the first of many to address the concept of premalignancy, reporting an association of ichthyosis (a white patch) preceeding epithelioma (neoplasia) in an elderly lady he had treated (6). Further cases were then reported of the association between an oral white patch and epithelioma, providing evidence of the premalignant nature of some oral keratoses (6,7,8). In 1877 oral "leucoplakia" was described and distinguished from psoriasis by Schwimmer (9). Today the term leukoplakia persists and continues to be used to describe a range of oral white lesions. Furthermore the historical recognition of malignant change associated with leukoplakia has been confirmed during the course of this century (10,11,12).

Descriptive terms for the oral keratoses are diverse (Table 1). The suspected aetiologies of leukoplakia were diverse in the early literature. Irritation from broken teeth, spicy food or drink, infection and tobacco use were often considered important (13,14,15). Syphilis was more commonly considered to be causative (13,16,17,18); this association was endorsed especially during the first decade of the 20th Century in France (16). Initially, dental filling materials were reported to be important aetologically in oral keratoses (11,13,14) but more recently doubt concerning this aspect has been raised (19). Alcoholic beverages especially spirits, were considered to exert an effect on the oral mucosa (20). This is now reported to be an aetiological component of oral squamous cell carcinoma (21).

TABLE 1

Synonyms and Descriptions Used During the Last Century for Oral Keratoses

Leucoplakia

Leucokeratosis

Leukoplakia

White tablets

Plaques des fumeurs

Tylosis linguae

Psoriasis linguae

Ichthyosis

Leucoma

Ichthyosis linguae

Smokers' patches

Leucoplasie

Plaques de la bouche

Plaques opalines

Hyperkeratosis buccalis vel linguae

Some observers considered the different forms of tobacco use to exert a greater or lesser effect on the mucosa, while Bloodgood (1925) stated that the quantity of tobacco used was the critical feature in leukoplakia (22), Bettman (1931) stated that he had never "seen leukoplakia in a patient who had not used tobacco" (23). Indeed Hollander (1933) included tobacco as first in a list of local factors important in understanding leukoplakia (24).

Other local factors have been associated with leukoplakia: galvanic effects due to dissimilar metal restorations, corrosion products and food allergies (25-29). Dental prostheses of poorly cured vulcanite were thought to encourage chronic bacterial growth and cause leukoplakia; the mercury sulphide of overcoloured vulcanite and sulphur in undercured dentures were also thought to cause additional mucosal irritation (25). Chronic oral sepsis and bacterial infections of the gingivae were also reported to be important (27).

Leukoplakia was reported to involve other mucosal sites such as the vagina, oesophagus and bladder (24). Although local low grade sepsis was considered to be aetiological, investigators reported systemic aspects associated with leukoplakia. Diabetes and Vitamin A deficiency were suspected of encouraging leukoplakia, vitamin A exerting its effect alone or in association with Indian betel nut chewing (30-32).

Oral leukoplakia has been reported to be associated with ichthyosis, lichen planus and psoriasis of the skin (26). Further cases were quoted by Fitzwilliams (1927) of leukoplakia associated with lupus

vulgaris and oral tuberculosis (26). Indeed Fox (1925) stated that opinions differed regarding features of leukoplakia and that "nobody has yet defined leukoplakia satisfactorily and different observers have no doubt described widely different lesions" (27). He went on to indicate in his paper describing clinical and histological features of leukoplakia, that the term leukoplakia may encompass different entities (27).

Saison (1871) reported diagnostic problems in differentiating between leukoplakia and lichen planus of the buccal mucosa: in one case, the oral lesions of lichen planus were not accompanied by skin eruptions (33). Later, in 1885, Thibierge discussed further diagnostic problems in differentiating types of oral keratosis including leukoplakia, smokers' patches, local trauma, buccal psoriasis, marginal exfoliative glossitis ("la glossite exfoliatrice marginee"), oral syphilitic lesions and lichen planus (34).

Mucosal involvement with lichen planus was described by an increasing number of authors by the late nineteenth century (34). It was a keratosis distinguishable by clinical presentation. However this was not infallible as illustrated by the growing discussion on oral keratotic diseases (6-8).

Lichen planus is a chronic mucocutaneous dermatosis (12). Clinicians were aware of cutaneous lichen planus by the early 18th century and a wealth of descriptive terms arose during the next century to specify variations of the disease (35-38). Oral involvement however was not reported until the latter half of the 18th century (38,39). The mucosal lesions had a similar appearance to the papular skin lesions but were flat. It was during an address by Wilson in 1866 to the 34th annual meeting of the British Medical Association, that he attempted to simplify the terminology for cutaneous lichen planus (38). The variety of synonyms and descriptive terms in use then are shown in Table 2.

Pospelow (1881) described to the Medical Society of Moscow the manifestations of lichen planus on the tongue, palate, gingivae and the associated cutaneous eruptions in a twenty three year old student (40). In a later review of cutaneous and mucosal lichen planus, Thibierge (1885) commented that the lichen planus papules affecting the mucosa had atrophic centres and were "un peu gros qu'un grain de millet..." (34,40). An early French thesis by Lavergne (1883) on lichen planus detailed observations by Neumann (1881) and Crocker (1882) of lichen planus involving the mouth (41-43). These authors documented the clinical appearance and history of oral lichen planus in its morphological forms. Indeed, Thibierge (1885) illustrated a case of cutaneous lichen planus that was preceeded by the development of oral leukoplakia "cette plaque qui presente absolument les characteres d'une plaque de leukoplakie de la joue" (34). This case presented with unilateral leukoplakia of the commissure, buccal mucosa and gingivae extending to the throat (34). These features were

TABLE 2

Historical Synonyms and Descriptive Terms

for Cutaneous Lichen Planus

Lichen ruber planus

Lichen ruber accruminatus

Lichen moniliformis

Lichen obtusus

Lichen planus erythematosus

Lichen planus atrophicus

Lichen planus sclerosus

Lichen planus morphocious

Lichen planus striatus

Lichen ruber of Hebra

Lichen accruminatus of Kaposi

Pityriasis rubra piloris of Devergie

atypical for a patient with a history of only a few months pipe smoking and the ensuing cutaneous lichen planus may suggest a diagnosis of oral plaque type lichen planus (34). Lavergne (1883) had not identified oral lesions of lichen planus but recognised that other investigators including Crocker (1883) had reported oral involvement of the condition (39,41). Wickham (1895) described striae, a pathognomonic sign of lichen planus (44): Wickham referred to the striae seen in cutaneous lichen planus and did not describe striae in the oral forms of lichen planus.

The pharyngeal involvement of lichen planus was considered by Wilson (1866) as a cause of marasmus, a wasting condition further described by Hebra (1869) (36,38). Later Crocker (1883) reported examples of lingual, buccal and pharyngeal lichen planus (39).

Pathological features of cutaneous lichen planus were initially described by Hebra and Kaposi (1880) and were already becoming the subject of communication (45,46). It was not until Dubreuilh's report in 1906 that the pathological features of cutaneous lichen planus and oral lichen planus were compared (47). The microscopic features upon which diagnosis of lichen planus was based, closely resemble those currently in use today (48,49). Dubreuilh (1906) considered a biopsy of the oral mucosa to be easier than that for skin as "la muqueuse est moins sensible que la peau et l'on ne fait pas de cicatrice" (47). This opinion was endorsed by Dubreuilh's observation that lichen planus occurred more frequently on the buccal mucosa than on the skin (47). Important pathological features recognised by Dubreuilh included a subepithelial dense and uniform infiltrate of small

lymphocytes, oedema of the basal layer of epithelial cells and an absence of plasma cells and mast cells (47).

The clinical presentation of oral lichen planus encompasses a variety of morphological variations. Wilson (1866) described the appearance of "round white spots, having the normal size of the papules on the skin, but without elevation" on the tongue, buccal mucosa and labial mucosa of a 56 year old female patient (38). Pospelow (1881) similarly described flat papules on the tongue of a 23 year old male patient (40). Thibierge (1885) also described a case of irregular plaques and papules of oral lichen planus occurring on the buccal mucosa adjacent to a grossly carious molar (34).

Crocker presented an encompassing appraisal of lichen planus to the Dermatological Society of London in 1900 (50). In discussion, Dr Pringle considered the commoner sites of oral lichen planus to be palate and buccal mucosa, rather than the tongue. Dr T. E. Fox recounted a French investigator who considered that "in two thirds of the cases the eruption was seen in the mucous membrane". He disagreed with the frequency but observed "that the eruption in the mouth does not itch and, therefore, it very often passes unnoticed" (50).

Initial observations of malignant change in oral white patches were made by Sir James Paget (1851), Morris (1874) and Clarke (1874) (4,6,7). In France Hallopeau (1910) recorded an association of neoplasia with gingival lichen planus and recommended that a biopsy was valuable in such a case (51). Copinger (1883) described ichthyosis of the tongue with malignant degeneration (52).

Microscopic sections were shown to be a useful adjunct in determining the malignant changes (52). Previously Fox (1874), adding to the discussion concerning ichthyosis and keratosis linguae, recognised microscopic features of altered epithelium of a keratotic tongue and stated, in referring to ichthyosis linguae, "the latter, no one I suppose now doubts, is an early stage of epithelioma..." (53). Sturgis and Lund (1934) reviewed aetiological aspects for leukoplakia and while outlining local and systemic aspects, considered that the persistent keratotic lesion remained an indicator of possible malignant degeneration (54).

Current thinking is that the majority of white lesions of the oral mucosa are the result of altered keratinization, with the remainder due to secondary factors directly affecting the epithelial cells and keratin (for example an aspirin burn). The former lesions are commonly considered to comprise a group of "Keratoses" which include conditions or lesions such as lichen planus, leukoplakia and discoid lupus erythematosus. The present thesis is largely concerned with the study of oral lichen planus and leukoplakia which are the two most common types of oral keratoses. As this was a prospective study of unrelated keratoses, a small number of less common keratoses were included.

CLASSIFICATION OF ORAL KERATOSES

Diseases may present with a great spectrum of features. Diagnosis when defined as the "determination of the nature of a case of disease", enables not only treatment appropriate to the disease but also permits classification of disease (55). Diseases may be recognised by changes in the function and structure of the tissues and as discussed by Underwood (1981), the diagnostic process requires a comparison of features of disease and normality (56). Understandably, there are many aspects that influence each diagnostic process. The scientific basis for oral diagnosis has been recently comprehensively reviewed by Dabelsteen and MacKenzie (1985) (57). Disease classification on the whole provides a useful and systematic arrangement of disease entities based on differing characteristics (56).

The main emphasis in this thesis is related to differences between lichen planus and leukoplakia and reference is made to the other keratoses only where they are relevant to the study of these two conditions. The main groups of disease included under the term "oral keratoses" are listed below:

Genetic and Developmental - oral epithelial naevus

Traumatic - frictional and smokers keratosis

Infective - candidiasis

Dermatological - lichen planus

Idiopathic - leukoplakia

Neoplastic - squamous cell carcinoma

Lichen Planus

Lichen planus is a distinctive mucocutaneous disease presenting with a variety of morphological features. A systematic classification of clinical oral lichen planus has been considered by numerous investigators. Classification according to aetiology, clinical appearance and treatment have been used and aspects of classification shall now be examined.

In the 19th and early 20th centuries papular, reticular and plaque forms of oral lichen planus were reported (34,38). At the turn of the century a bullous form of lichen planus was described by Poor (1905) (58). The use of histopathology to identify typical features of biopsy material of oral mucosa helped define the clinical range of lichen planus (47). Indeed it was considered that the oral biopsy was helpful in determining the diagnosis of cutaneous lichen planus (59). Fordyce et al (1919) included oral lichen planus in their assessment of clinical types of lichen planus (60). Similarities in clinical presentation with lupus erythematosus and leukoplakia were noted for the oral involvement (60). Additionally, White (1919) described oral papules and large plaques in a patient with lichen planus (61).

Culver (1920) endorsed the value of oral examination in doubtful cases of cutaneous lichen planus where "white lace-work eruptions so characteristic of lichen planus" may be identified on the oral mucosa (62). Tompkins (1952) suggested separating two main groups of lichen planus: a generalised form and a localised form (63). He considered that the generalised form presented with acute onset and rapid spread

of cutaneous and mucosal lesions, but carried a better prognosis as it had a shorter duration; the localised form followed a "chronic course with slower spreading of lesions..." (63). Within this latter group Tompkins included those with mucosal lichen planus "and all these carry a poor prognosis as to duration" (63).

During the latter half of this century four authors have put forward useful categorisations of the clinical types of oral lichen planus (Table 3). Cooke (1954) described lichen planus with eight morphological types of oral mucosal lesion (64). Andreasen (1968) described six morphological types, simplifying the keratotic types to papular, plaque and reticular and excluding the pigmented type of lichen planus (48). Shklar (1972) described four types; the first being papular variations that would present a range of keratotic lesions from linear to plaque-like (65). Shklar made bullous and atrophic lichen planus into the third and fourth types in his classification of lichen planus (65). The erosive type described by Shklar differed from the group of atrophic, erosive and ulcerative types described by Cooke (1954) and Andreasen (1968) (48,64,65). Tyldesley (1974) simplified the clinical presentation of lichen planus into three groups (66). The first group includes all the keratotic variations of lichen planus, the second group applies to the erosive variety, and the third group to a major erosive variety that may relate to the bullous types described by Cooke (1954), Andreasen (1968) and Shklar (1972) (48,64,65). The World Health Organization International Disease Classification (Dental Application), WHO.IDC.DA, has numerical codes for the disease types; these have however been restricted to five types - 697.00 to 697.04 (3). They relate to the

types as illustrated by Table 3 and bear a close relation to the types described by Andreasen (47).

Cooke, Andreasen and Shklar used their classifications to confer morphological differences seen in the disease (48,64,65). Tyldesley (1974) however, using a term adopted from Lehner's description in 1968 of recurrent oral ulceration, distinguishes widespread and rapid onset of ulceration from the other milder forms of lichen planus (66,67). This classification is similar to the interpretation Tompkins made in 1952 (63) and may be useful in the clinical management of oral lichen planus.

Authors have regarded desquamative gingivitis as a manifestation of lichen planus when it is associated with oral or cutaneous lichen planus (68,69). There are however other dermatoses that may be associated with desquamative gingivitis (69). It would appear that the presence of desquamative gingivitis alone is not diagnostic of lichen planus.

For the purposes of describing the clinical features of oral lichen planus in this thesis, Andreasen's categorisation will be adopted (48). The clinical appearances of oral lichen planus and oral leukoplakia will be examined next.

TABLE 3

Categorisation of Oral Lichen Planus

1954	1968	1972	1974	1978
AUTHOR				WHO.
Cooke	Andreasen	Shklar	Tyldesley	IDC.DA
DESCRIPTION				
1 linear				
2 discrete papular	1 papular	1 papular	1 non erosive	1 697.00
3 confluent papular	2 plaque			2 697.04
4 reticular	3 reticular			3 697.01
5 annular				
6 pigmented				
7 bullous	4 bullous	2 bullous	2 major erosive	4 697.03
8 atrophic erosive ulceration	5 atrophic 6 erosive ulceration	3 atrophic 4 erosive	3 atrophic and minor erosive	5 697.02

This table represents the author's interpretation of types of lichen planus. See text for explanation.

i) PAPULAR (WHO.ICD.DA 697.00). Papules on the oral mucosa are small, less than one millimetre in diameter, slightly raised and white. Clusters of papules may form lines producing a reticular appearance. The papules are most frequently seen on the non keratinizing mucosal sites. Occasionally non elevated papules may signify early lichen planus (49). This is illustrated in Figure 1 where papules on the buccal mucosa are present together with reticular lichen planus.

ii) PLAQUE (WHO.ICD.DA 697.04). White plaques, often irregular in texture and outline, can be seen most often on the dorsum of the tongue and buccal mucosa. Reticular and papular elements can surround the plaques. In the absence of these typical features of lichen planus a clinical misdiagnosis of leukoplakia may easily be made. Plaque lesions are more common in smokers. Variation in the outline of these plaques has been shown to occur within a four week period (48). An example of plaque lichen planus involving the dorsum of the tongue is illustrated in Figure 2.

iii) RETICULAR (WHO.ICD.DA 697.01). This is the most common form detected on oral mucosa (49). Fine white lines, slightly raised, spread irregularly over the mucosa, making a lace like pattern. Papules can be seen at the periphery of the migrating lace patterns (49). Buccal mucosa is most commonly affected (49). The lateral and ventral surfaces of the tongue can also manifest the reticular patterns. Andreasen (1968) described the pattern varying as the

FIGURE 1. Papular and Reticular Lichen Planus.

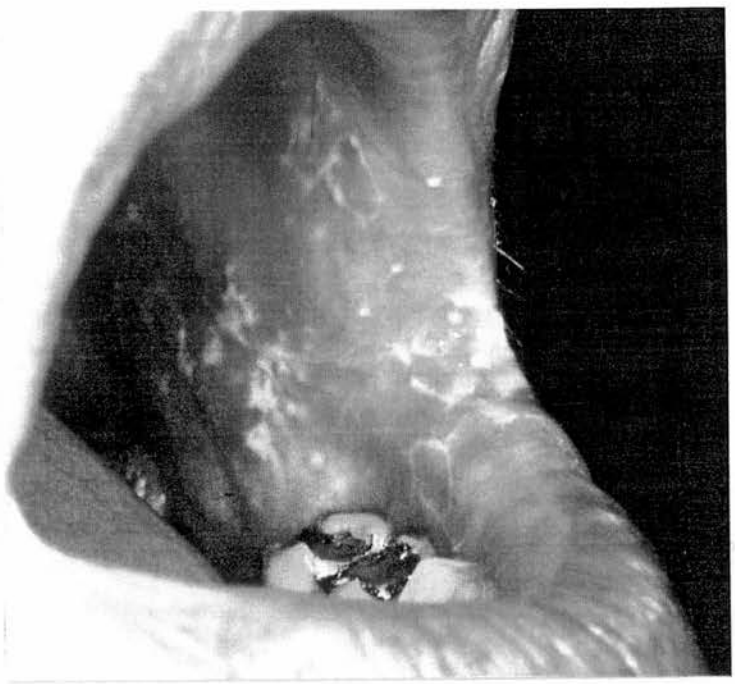
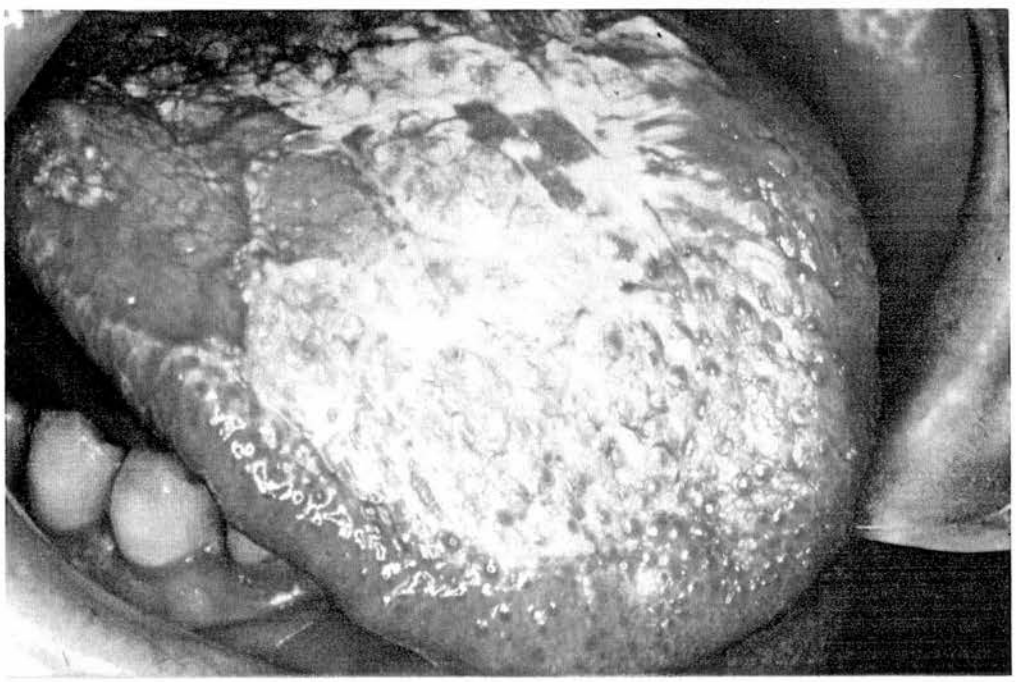


FIGURE 2. Plaque Lichen Planus.



disease progressed (48). Figure 3 illustrates reticular lichen planus involving the buccal mucosa.

iv) ATROPHIC (WHO.ICD.DA 697.02). Atrophic lichen planus appears as an erythematous area, the periphery of which is quite often made up of the reticular pattern. The redness is due to epithelial atrophy and frequently erosions will be seen. Again, the buccal mucosa is most frequently involved (48,49). Figure 4 illustrates atrophic lichen planus involving the hard and soft palate.

v) EROSIVE (WHO.ICD.DA 697.02). This form usually occurs in sites of atrophy, however discrete erosive lesions may exist singularly. The tongue and buccal mucosa are commonly involved. Tyldesley (1974) found it useful to classify minor and major erosive forms of lichen planus (66). The major erosive lesions affected four patients out of 115 and appeared with sudden and widespread, painful ulceration of the oral mucosa. The minor erosive form of lichen planus had a chronic clinical history, and included the clinical variants of erosive, atrophic, ulcerative and bullous lichen planus. The justification of this category was based on the similarity of histological and clinical symptoms (66). Erosive lichen planus involving the lateral and ventral surfaces of the tongue is illustrated in Figure 5. Note additional involvement of reticular lichen planus on the dorsum of the tongue and the buccal mucosa.

FIGURE 3. Reticular Lichen Planus.

FIGURE 4. Atrophic Lichen Planus.

FIGURE 5. Erosive and Reticular Lichen Planus.

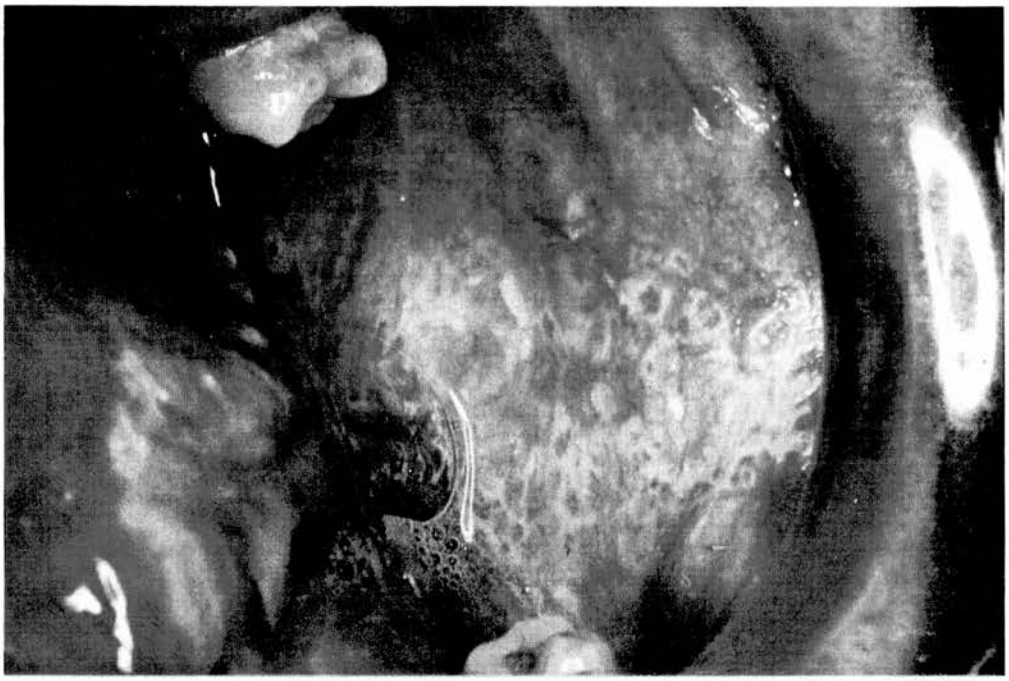


FIGURE 6. Bullous and Erosive Lichen Planus.

vi) BULLOUS (no WHO.ICD.DA number). Bullous lesions are the most rarely encountered manifestation of lichen planus (65). The bullae are difficult to identify: the epithelium is easily removed by normal masticatory forces from the lamina propria leaving large erosive lesions. While bullae are infrequently observed intact, the combination of bullae and erosive mucosa is illustrated in Figure 6.

vii) DESQUAMATIVE GINGIVITIS (WHO.ICD 523.13). This variant of the erosive lichen planus involves the free and attached gingivae (69). White reticular lines may also be seen clearly on the gingivae in affected patients (68). The erosive variant of desquamative gingivitis may be difficult to diagnose. Accompanying mucosal involvement with lichen planus often substantiates a diagnosis of lichen planus. Histological confirmation of desquamative gingivitis is often difficult especially since non-specific plasmacytic gingival inflammation is reported to complicate the histological picture (69). Other dermatoses, including pemphigus vulgaris, bullous pemphigoid and cicatricial pemphigoid may also have an associated desquamative gingivitis (68,69).

viii) CUTANEOUS LICHEN PLANUS (WHO.ICD 697.0). This is variable in its occurrence with oral mucosal lesions. Tompkins (1952) reported cutaneous involvement in 37% of his group of patients with mucosal disease (63). Oral lichen planus may preceed any cutaneous eruption and Dubreuilh (1906) was reported to have stated that, in his experience, mucous membrane was more frequently affected without skin than is skin without the mucous membrane involvement (47). Table 2 illustrates the numerous terms that have been used in describing the

clinical presentation of lichen planus. The essential clinical feature of a cutaneous lichen planus lesion is a papular, slightly erythematous lesion with a flat surface, often with grey white striae (Wickham's striae) (44). This is illustrated in Figure 7 where cutaneous lesions involve the dorsal surface of the hand. Other sites susceptible to cutaneous lichen planus include flexor surfaces of wrists and anterior surfaces of legs (36). Additionally, sites of previous or recent trauma may also produce an isomorphous reaction known as the Koebner phenomenon (66,69). Cutaneous lichen planus resolves in almost 90% of patients within two years; however relapse is common (20%) and melanotic pigments may persist in the lesion site (70-72).

The morphological patterns of cutaneous lichen planus are broadly similar to oral mucosal variations of lichen planus. There are follicular, linear, annular, hypertrophic, atrophic and bullous type lesions (64). These may be usefully viewed as having a white or ulcerative presentation as previously described for the classification of oral lichen planus. While cutaneous lichen planus is variably associated with oral mucosa lichen planus, for the purposes of this thesis the cutaneous form shall be referred to only to elucidate the discussion of the oral disease.

FIGURE 7. Cutaneous Lichen Planus.

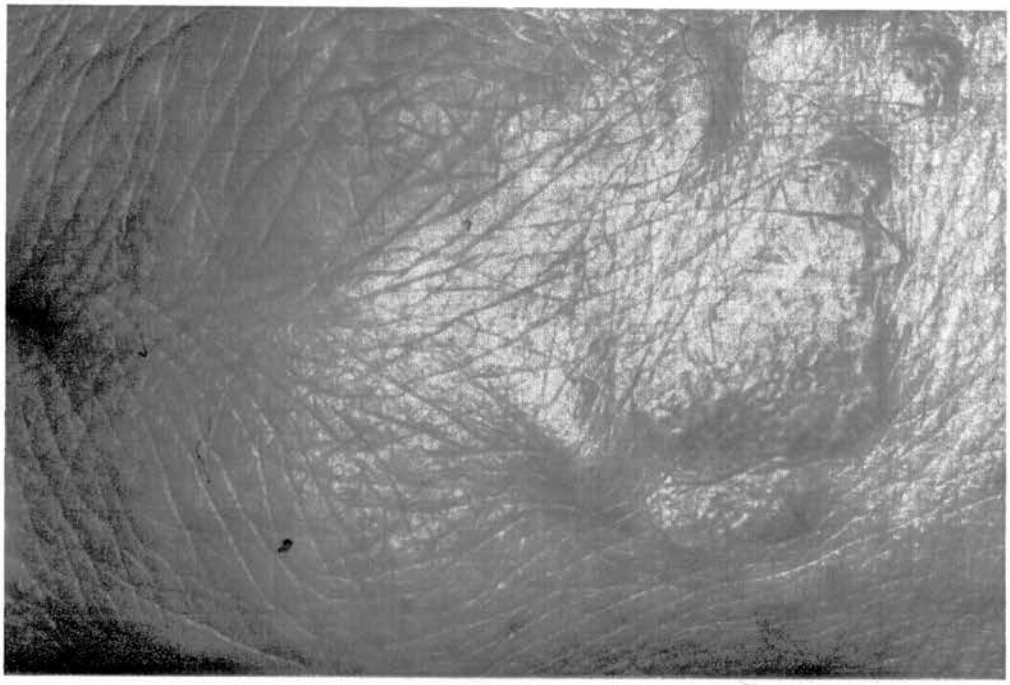


FIGURE 8. Homogeneous Leukoplakia.

Leukoplakia

In the past the term leukoplakia was used to describe any white patch in the mouth, but it now has a more restricted meaning. The WHO definition (1978) defined leukoplakia as a white patch which cannot be removed by scraping and cannot be attributed to any other diagnosable disease (3). More recently this definition has been altered in view of the possible effects of tobacco on the oral mucosa, so that while the term leukoplakia should not be associated with lesions resulting from known physical or chemical trauma, if a white lesion is thought to result from the use of tobacco it can still be described as leukoplakia (73).

Leukoplakia is characterised clinically by the presence of single or multiple white patches of the oral mucosa. They may be distinctly circumscribed or merge with the surrounding mucosa (74). Although the original WHO definition required the white patch to be five millimiters or greater and this definition was used by some authors (74), the use of size for this diagnosis was subsequently dropped (3). The surface texture may be smooth, fissured, or nodular (75). The colour of the lesion varies also, depending on the patient habits, mucosal site, keratin thickness and degree of erosion and atrophy of the lesion. Commonly, grey white or yellow white is seen, however tobacco use may stain the lesion brown (76). Leukoplakia has an International Disease Classification coding of IDC.DA.528.6X (3). The broad morphological types of leukoplakia are homogeneous, speckled and nodular and combinations of these appearances may be seen (72-84).

Clinical Appearance of Leukoplakia

i) HOMOGENEOUS LEUKOPLAKIA (WHO.ICD.DA.529.6X). This appears as an irregular white area of mucosa with a surface that may be smooth, fissured or raised. There may be a defined margin or the lesion may merge into the surrounding mucosa. Where there is marked hyperkeratosis, a plaque type lesion may be seen. The term verrucous leukoplakia is used where marked hyperkeratosis occurs producing an irregular or papilliferous surface (84). Homogeneous type lesions are illustrated in Figures 8 and 9 involving the ventral surface of the tongue and the buccal mucosa respectively.

ii) SPECKLED LEUKOPLAKIA (WHO.ICD.DA.529.6X). This presents with erythematous areas and white keratotic areas of mucosa. The keratotic areas appear granular or as small white plaques. The erythematous base is considered to be erythroplakia and the clinical entity may also be described as speckled erythroplakia (85-86). A combination of homogeneous and speckled leukoplakia is seen on the mucosa involving the floor of mouth and ventral surface of tongue in Figure 10.

iii) NODULAR LEUKOPLAKIA (WHO.ICD.DA.529.6X). This presents with keratotic nodules, which are excrescences, irregularly scattered on an erythematous base that merges into the adjacent normal mucosa. The nodules vary in size however but do not usually exceed three to four millimeters in diameter. They may appear a few millimeters raised from the surface of the mucosa.

FIGURE 9. Homogeneous Leukoplakia.

FIGURE 10. Homogeneous and Speckled Leukoplakia.

iv) CANDIDAL LEUKOPLAKIA (WHO.ICD.DA.529.72). This commonly appears as a plaque or speckled keratotic lesion affecting the buccal or commissure mucosa. Diagnosis is reliant on histopathological staining to reveal the spores and hyphae of the candidal organism. The aetiological significance of the organisms is speculative, as discussed later. It is recognised that this type of leukoplakia may be impossible to distinguish clinically from speckled leukoplakia.

The association of leukoplakia with other white lesions of the mucosa is poorly documented in the literature. Differences in categorisation of leukoplakia into the types specified above have existed between authors throughout this century. Thoma (1950) classified oral leukoplakia in four groups according to the degree of keratinization and associated monocytic infiltrate, thereby the microscopic appearances contributed to the categorisation of the lesion (20). The grade (I) leukoplakia described the initial reaction of the mucosa to irritation showing a clinically erythematous area. Grades (II) and (III) were leukoplakias with smooth or wrinkled white patches, the grade (III) being more advanced and quite obvious in the mouth "even by the layman" (20). The lesions were reported to frequently affect the tongue and the buccal mucosa. The grade (IV) was "often called neoplastic leucoplakia" and showed papillomatous or verrucous changes (20). Ulceration and restriction of movement were futher features of this grade of leukoplakia and neoplasia was heralded with induration and "down growths of epithelium" (20). This classification has been superseded in clinical practice by the WHO International Classification for Disease terminology as described above (3).

EPIDEMIOLOGY OF ORAL KERATOSES

Prevalence studies relate to the amount of a disorder existing in a chosen population at a stated time (87). In addition to the provision of health care, prevalence studies assist in understanding "the natural history of disease" (88). Axell (1976) carried out a prevalence study of oral mucosal lesions in an adult Swedish population (74). The keratotic lesions comprised a large proportion of the lesions recorded. If lesions of lichen planus, pre-leukoplakia, leukoplakia, nicotinic palate, snuff dipper's lesion, frictional keratosis, cheek and lip biting, discoid lupus erythematosus and white sponge naevus are cummulated, oral keratoses were detected in 31.95% of the population examined (74) (Table 4). It must be noted however that the figure of 31.95% includes the nebulous entity of "pre-leukoplakia" which is of doubtful clinical significance. Also the extraordinary high incidence of "snuff dipper's lesion" can only be explained on the basis of the selected nature of the adult Swedish population.

Prevalence of Oral Keratoses

Lichen Planus

Lichen planus is reported to have a prevalence in the Swedish adult population of 1.89% (74). Females are more commonly affected, 2.19% compared to males 1.59%. The inclusion of lichenoid type reactions which are tissue responses to medications and seen to subside after cessation of the particular medication, increased the prevalence

TABLE 4

Prevalence Study of Oral Mucosal Lesions in an Adult Swedish Population (20,333 Individuals)

	Percentage
Lichen planus +	(1.89)*
lichenoid eruptions + atrophy of tongue	2.20
Preleukoplakia	6.35
Leukoplakia	3.60
Nicotinic palate	1.12
Snuff Dipper's lesion	8.04
Frictional keratosis	5.47
Cheek and lip biting	5.14
Discoid lupus erythematosus	0.01
White sponge naevus	0.02
Total (%)	31.95

After Axell (1976) (74)

* Value for lichen planus only

to 2.20% (74). Earlier studies on special populations, provided varying prevalence values for lichen planus (74). Pindborg et al (1965a,1965b,1966) in three studies on the Indian subcontinent reported a prevalence of between 0.02 and 0.22% for oral lichen planus (89-91).

Table 5 illustrates the range of values that different investigators obtained from both general and special populations (48,89-102). Hellier (1940), McCarthy (1941) and Arndt (1971) noted oral lichen planus in patients attending dermatological clinics (95,96,102). The prevalence remains between 0.1 and 1.7% with McCarthy recording the highest value. Studies have been carried out in Europe, the Indian subcontinent and North and South America (48,89-91,98,100). No obvious differences in prevalence appear to exist between major geographical locations.

Oral lichen planus may be concommitant with cutaneous lichen planus (2,72). Dermatology clinics have provided substantial numbers of patients for such investigations and generally oral lichen planus is seen in up to 77% of patients with dermatological lichen planus (72,103). Hellier (1940) recorded between 0.1 and 1.25% of dermatology outpatients to have oral lichen planus (95), 0.442% in 676373 dermatological patients were calculated by Arndt in 1971 to have dermatological lichen planus (96). The prevalence of cutaneous lichen planus in patients attending with oral lichen planus is variable. Andreasen (1968a) reported 44%, Cooke (1954) 10% and Tyldesley (1974) 35% of patients with both oral and cutaneous involvement of lichen planus(48,64,66).

TABLE 5

Prevalence of Lichen Planus

%	Group Investigated	Number	1st Author	Date	Ref.
0.02	Indian Dental Attenders (IDA)	10000	Pindborg	1966	91
0.14	Dermatology patients	32879	Rufenacht	1968	99
0.19	(IDA)	10000	Pindborg	1965a	89
0.22	(IDA)	10000	Pindborg	1965b	90
0.28	Male 20 year olds	2174	Borghelli	19741	100
0.3	General population	50915	Mehta	1971	93
0.442	Dermatology outpatients	676373	Arndt	1971	96
0.6	Dental clinics	-	Shklar	1972	65
0.6	Textile workers	57518	Smith	1975	98
1.1	45-97 year old soldiers	785	Bhaskar	1968	101
1.2	General population	-	Moschella	1975	94
1.25	Dermatology outpatients	-	Hellier	1940	95
1.5	Indian villagers (Kerala)	7639	Pindborg	1972	99
1.7	Dermatology patients	927	McCarthy	1941	102
1.89	General population	20333	Axell	1976	74

Leukoplakia

The prevalence of leukoplakia in general and special populations has been well documented. Numerous studies in Europe and India of general populations show a prevalence of leukoplakia of 2 to 4% (table 6)

In three surveys by Pindborg et al (1965a,1965b,1966) that recorded mucosal lesions of patients attending dental schools in different areas of India, a prevalence of 1.55 to 3.28% was noted for leukoplakia (89-91). Similarly, other surveys recorded prevalence values ranging between 1 and 4% in special populations(104-107) (Table 6). Increased prevalence values of 11.7% are recorded where an older and predominantly male population is examined as Smith et al reported in 1975 (98). Indeed a prevalence of up to 54.1% has been recorded in East European industrial workers but the reason for this high percentage is unclear(108,109). Patients younger than twenty years are infrequently found to have leukoplakia (81-82). Axell (1976) recorded prevalence of 6.2% for a 55 to 64 age group and a lower prevalence of 4% for those patients a decade older (74).

TABLE 6

Prevalence of Leukoplakia

%	Group Investigated	Number	1st Author	Date	Ref.
1.3	Danes >65yrs	557	Gabrowski	1974	107
1.55	(IDA)#	10000	Pindborg	1965a	89
1.7	Indian	50915	Mehta	1971	93
2.1	25-34 year olds*	-	Axell	1976	74
2.36	Indian	5000	Zachariah	1966	105
2.84	(IDA)#	10000	Pindborg	1965b	90
3.0	Denture wearers >65yrs	201	Chrigstrom	1970	106
3.28	(IDA)#	10000	Pindborg	1966	91
3.53	European	20333	Axell	1976	74
3.6	European	5613	Bruszt	1962	104
4.0	65-74 year olds*	-	Axell	1976	74
6.2	55-64 year olds*	-	Axell	1976	74
11.7	Indian males >35yrs	57518	Smith	1975	98
12.0	Industrial workers	-	Chiechanovicz	1968	108
54.1	East Europeans	-	Smoljar	1971	109

Indian Dental Attenders from different regions in India.

* The population studied by Axell (74) consists of these sub-populations according to age.

Sex Prevalence of Oral Keratoses

Lichen Planus

Oral lichen planus is commonly reported to involve females more frequently than males (59,61,62,68). However dental clinic attendance patterns may influence the ratio. Pindborg et al (1972) found no difference in prevalence of lichen planus between sexes. Evidence that males are predominantly affected with lichen planus is also reported (110,111).

Leukoplakia

Oral leukoplakia historically was reported to be a predominantly male disease (20). During the present century, this feature has altered from a reported male to female ratio of 19:1, to 1:1 (20,81,82). Factors reported to be associated with this changed ratio include increased tobacco use by females (112). Examples of local tobacco customs such as reverse cigarette smoking, seen in the women of Andhra Pradesh and snuff use in the Southern States of America are reported to be associated with an altered sex ratio (113,114,115).

Site Prevalence in Oral Keratoses

Lichen Planus

Lichen planus has been reported to involve all oral sites (48,49). Most commonly affected is the buccal mucosa, Andreasen (1968)

reporting such involvement in 114 patients out of the study number of 115 patients (48). The buccal site adjacent to the position of a standing third molar tooth is reported to be most frequently involved with lichen planus. The least commonly affected site reported was the upper vermilion border of the lip (66)(Table 7).

Leukoplakia

Leukoplakia may involve any oral site but no particular site is predominantly affected (81,82). Waldron and Shafer (1975) found the mandibular alveolar mucosa and sulcus followed by the buccal mucosa to be the most commonly affected (82) (Table 8). Simpson (1957) found the buccal mucosa to be most commonly affected, with the tongue more frequently involved in males than females and the floor of mouth more frequently involved in females than males (116). The tongue, alveolus, lips and palate were individually less frequently involved than buccal mucosa but when these sites were considered together they comprised the largest proportion of leukoplakias (116). As with other studies patients were seen to have lesions in more than one site (116). Thoma (1950) reported the tongue as being the most common site affected, this being often associated with tertiary syphilis (20,117). Silverman (1968) reported on the clinical aspects of leukoplakia in 117 patients and found the buccal mucosa was most commonly affected, more so in men (20%) than in women (6%)(118). He also reported leukoplakia to be four times more prevalent on the tongue in denture wearers than in non denture wearers (118).

TABLE 7

Site Location of Lichen Planus

in 115 Patients

Site	Number*
Buccal mucosa	114
Tongue: dorsum	44
Tongue: lateral and ventral surface	40
Buccal gingivae (mandible)	39
Vermilion border (lower)	24
Buccal gingivae (maxilla)	23
Hard palate	23
Palatal gingivae	11
Labial mucosa (lower)	9
Lingual gingivae (mandible)	9
Floor of mouth	9
Labial mucosa (upper)	7
Vermilion border (upper)	3

From Andreasen (1968) (48).

* Number of patients.

TABLE 8

Site Location of Leukoplakia in 650 Cases

Site	Percentage
Mandibular mucosa and sulcus	25.1
Buccal mucosa	21.9
Palate	10.7
Maxillary mucosa and sulcus	10.7
Lips	10.3
Floor of mouth	8.6
Tongue	6.8
Retromolar	5.9

From Waldron and Shafer (1975) (82).

Malignant Potential of Oral Keratoses

Oral keratoses have been observed to undergo neoplastic change (5-14,75,119-122). Early investigators, observing pathological changes in keratoses that apparently heralded neoplastic change, saw an opportunity of reducing the morbidity and mortality of oral cancer through identification of the premalignant lesion (13). The term 'leukoplakia' became associated, non specifically, with precancerous keratotic lesions. Kerr (1958) addressed this problem, stating that "not all white lesions are precancerous; for that reason they should not all be designated 'leukoplakia'" (117). Lichen planus in plaque form may clinically feign leukoplakia when other identifying features are not available. This diagnostic problem remains today.

Lichen Planus

Oral lichen planus has been reported to undergo malignant transformation (123-126), Hallopeau (1910) providing one of the earliest descriptions of the occurence of malignant change in oral lichen planus (51). However, Krutchkoff et al (1978) and Kabani et al (1982) consider there to be insufficient evidence to regard lichen planus as a premalignant disease (127,128).

Malignant change recorded in oral lichen planus has been reported to be between 0.3 and 3% (123,125,127-133). Murti et al (1986) calculated a relative risk of 3.3 for developing cancer in oral lichen planus (133). Although this value did not appear statistically significant, an observation that cancer developed in younger patients

(average age 39) in lichen planus was made. This compared with an overall average age of 55 years for oral cancer patients in an earlier study by Mehta et al (1971) (93). Erosive and atrophic forms of lichen planus are thought to be more likely to undergo malignant change than other forms (123,132,133,134-137) and Holmstrup and Pindborg (1979) reported an association of erythroplakia and squamous cell carcinoma with lichen planus (126). Numerous investigators have published case reports of the occurrence of oral carcinoma in patients with oral lichen planus (126,127,138,139) and Pindborg (1980) in his evaluation of malignancy in oral lichen planus which included data published by Fulling (1973) and Holmstrup (1975), reported five patients out of 470 who had developed oral carcinomas within sites of lichen planus (123,140,141).

This malignant potential of oral lichen planus was further reviewed and accepted at an international seminar on oral erythroplakia and lesions related to tobacco habits, where lichen planus was considered as a probable precancerous condition (142). This was 24 years after Warin (1960) presented case reports of the development of carcinoma in oral lichen planus and noted that "..it has usually been taught that malignancy never develops in lichen planus of the mouth..."(143). Holmstrup et al (1988), in the most recently reported long term study of malignant change in oral lichen planus, concluded that oral lichen planus fulfills the WHO criterion of a premalignant condition, with a fifty fold increased risk of malignant change over that expected in the general population (140). 1.5% of cases were reported where squamous cell carcinoma arose in sites of oral lichen planus (144). The study comprised 611 patients (409 Female, 202 Male) followed for

periods from 1-26 years. The observed number of carcinoma cases was significantly higher than the estimated number expected to develop oral carcinoma in a general Danish population sample of similar size and age distribution ($p<0.00001$)(144).

Contrary views were presented by Krutchkoff et al (1978) and Kabani et al (1982)(127,128). Krutchkoff et al (1978) reviewed the world literature on malignant change in lichen planus and considered that only 15 of the 223 cases reported were substantiated by valid records of pre-existing lichen planus followed by neoplasia and concluded that there was insufficient evidence to confidently regard oral lichen planus as a premalignant condition (127,128).

The assessment of epithelial dysplasia may provide a method for determining malignant potential (145,146), but there are conditions in which epithelial dysplasia can be identified but does not necessarily imply a malignant potential (141). The occurrence of epithelial dysplasia in lichen planus has been examined (127-132,147,148) and Krutchkoff and Eisenberg (1985) put forward criteria for identifying "lichenoid dysplasia" with a view to sub-classification of lichenoid lesions of the oral mucosa (131). Kabani et al (1982) however suggested that lichen planus and lichenoid dysplasia are different diseases (128). While the criteria detailed by Krutchkoff (1985) provide description for both lichenoid dysplasia and lichen planus diagnoses (131), it is difficult to apply these criteria in the diagnosis of biopsies from individual patients. In addition, while Krutchkoff's aim "..to eliminate individual diagnostic variation based on training, experience, or idiosyncratic bias of the pathologist so

that diagnoses can be considered reliable and accurate.." is commendable, the system he has suggested perhaps will increase the numbers of diagnoses returned as lichenoid dysplasia and reduce those of lichen planus; without any major benefit.

There is however no general agreement among pathologists concerning the relationship between epithelial dysplasia and malignant change in lichen planus and it must always be remembered that the simultaneous occurence of lichen planus and squamous cell carcinoma in the same mouth may be a chance occurrence. Moderate or severe dysplasia seen in lichen planus has been suggested to carry increased risk of malignant change (129,134,137). A high proportion of moderate and severely dysplastic lesions of the tongue and floor of mouth for lichen planus and control studies were reported by Odukoya (1985)(129). This would suggest that certain oral sites may indeed carry greater risk for the development of epithelial dysplasia in chronic inflamatory conditions.

Leukoplakia

The malignant potential of leukoplakia has been widely documented and is generally accepted (80-82,149,150). Reported malignant transformation rates range from 0.8 to 9.9% for different populations (151,152). Centres vary in their diagnosis and management of leukoplakia and this is recognised as a possible influence on the transformation rates found (141). The follow up periods vary in the reports on malignant transformation, however the malignant transformation in a ten year period provides a useful gauge of the

disease. Mehta et al (1972) reported a study of 3674 Bombay policemen, of whom 117 had leukoplakia and one (0.9%) developed oral cancer in the ten year period of follow up (151). It was reported that almost all leukoplakias detected in chewers of tobacco regressed while persistent leukoplakias occurred in the smoking habit group. The one policemen who died from oral cancer had leukoplakia and he practised both "pan" chewing and "bidi" smoking (151).

Waldron and Shafer (1975) in their long term study (13 years) showed that the risk of developing epithelial dysplasia, carcinoma in situ or invasive carcinoma varied between the anatomical locations of leukoplakia (82). The high risk sites included the floor of mouth, the tongue and the lips where 43%, 24% and 24% of cases respectively showed epithelial dysplasia or carcinoma histologically. Furthermore where leukoplakia occurred with erythroplakia, the number of cases in which epithelial dysplasia or carcinoma was determined histologically, rose to 45-65% (141,150). Erythroplakia is a flat velvety red lesion that may occur singularly or in association with leukoplakia as speckled leukoplakia (85). While there did not appear to be significant differences in frequency of occurence of erythroplakia between male and female, there were more cases in the fifth decade or younger presenting with leukoplakia (39%) compared to erythroplakia (19%) (82). Mandibular, maxillary, tongue and floor of mouth mucosa was more frequently affected by erythroplakia in females while the lips, palate, buccal, and retromolar mucosal sites were more frequently affected in the males (82).

The evaluation and monitoring of the malignant potential of

leukoplakia is ultimately dependant on the microscopic changes observed in the epithelium (80,139). The subjective nature of this morphological evaluation has led to attempts to develop a system of photographic standardisation or computer aided analysis of features observed (146,149,153,154). More recently, morphometric methods of image analysis for evaluating epithelial cells and immunocytic infiltrates have been shown to be valuable in a more objective assessment of epithelial dysplasia in leukoplakia (150). As epithelial dysplasia may not singularly determine the malignant potential of the lesion (138,156,157), other parameters, notably immunological aspects, have been investigated in oral premalignant conditions and neoplasia (158,159). The value and implication of these investigations is unclear at present.

A review of the literature relevant to clinical and histological features of leukoplakia was available in 1980 following an international symposium on premalignancy (82,160). This has been supplemented by the work of Katz (1985) in which the Smith-Pindborg method of standardisation of epithelial dysplasia was performed on 214 cases in which the clinical features were known (161). For the idiopathic keratoses in the non keratinizing sites, floor of mouth and oropharynx, half the samples comprised severely dysplastic lesions while buccal alveolar mucosa and gingivae showed mild dysplasia in general (161). Although these features did not reach statistical significance, they convey the problem of evaluating dysplasia in oral tissues (161).

The problems encountered with studies of idiopathic keratoses include

the evaluation of malignant potential, prevalence of dysplasia, associated clinical and aetiological factors and the subjectivity of interpretation of all these aspects. Comparison of the study by Katz (1985) with that of Waldron and Shafer (1975) shows differences in male to female ratios and site incidence for dysplasia (82,161). Katz (1985) examined the distribution of 214 cases of "mild, moderate and severe dysplasia" and found no significant differences for race, sex or age (161). However lesions on the floor of mouth and oropharynx included 50% of the severe dysplasias (161). These features parallel Waldron and Shafer (1975) who found that 43% of biopsies from the floor of mouth showed epithelial dysplasia (82). Leukoplakias affecting the floor of mouth have been connected with increased risk of malignant change which may not neccessarily be related to the histological picture (10,162). In the United States where the topical use of tobacco is particularly prevalent, carcinoma is a distinctive feature in the site of tobacco placement (163).

At the 1980 symposium on premalignancy, the consideration of carcinomas arising from pre-existing lesions or (de novo) was put forward (164). Johnson (1980) was of the opinion that most cases of oral squamous cell carcinoma had no previous history of a long standing lesion, "perhaps as many as 80% appeared to be overtly invasive at presentation..." (164,165). Pindborg (1980) in discussion did not agree and considered that the "majority arose in altered tissue" referring primarily to idiopathic keratoses (164). Pindborg (1968) observed that dysplasia more often accompanied speckled leukoplakia and, in agreement with Shafer and Waldron (1975), considered that these lesions were more likely to become malignant

(82,163). Furthermore, after treatment of the candidal infections demonstrated in speckled leukoplakia by Jepsen (1965), the clinical appearance was seen to be improved (166).

It is useful to examine erythroplakia as this may occur in areas of oral leukoplakia or separately (84,86). The study by Shafer and Waldron (1975) showed that 91% of erythroplakia biopsies were histologically either invasive carcinoma, carcinoma in situ or severe epithelial dysplasia (82). The remaining 9% of biopsies were of a mild or moderate dysplasia (82). Follow up studies for erythroplakia have not been completed to date and thus the malignant potential cannot be commented upon. It remains a lesion with strong histopathological indicators of malignant change (167). Grading of epithelial dysplasia from mild to severe dysplasia and "carcinoma in situ" is seen to be variable between studies (147). The need remains for an internationally accepted set of criteria for evaluation of such epithelial dysplasia (147). Earlier work by Fischman (1982) demonstrated that the clinical diagnoses of "leukoplakia" or "hyperkeratosis" revealed frank carcinoma after biopsy in 13% of cases (148). This worrying aspect of clinical and pathological assessment is further complicated when we realise that the clinician already is aware that leukoplakia carries a malignant risk. The use of antifungal treatment of candidal leukoplakia has been initially promising in the reduction of dysplastic features (168).

AETIOLOGICAL FACTORS OF ORAL KERATOSES

a. Genetic Factors

Lichen Planus

Genetic predisposition to lichen planus may be shown by the occurrence of lichen planus in familial cases. As families may occupy a similar environment overall, the effect of infective or allergenic agents has also to be considered in such cases. A number of familial occurences of lichen planus have been reported (169-171). The majority of these cases are cutaneous; there appears to be only one report of oral lichen planus existing within twins (172). In these twins, oral and cutaneous involvement was extensive (172,173). Overall, the familial occurrence of oral lichen planus, indicating a genetic predisposition, is scant. The human transplantion antigen system, commonly called the human lymphocyte antigen (HLA) system, enables histocompatibility typing. Initial studies into the frequency of certain loci have shown that HLA-A28 has an increased frequency amongst non diabetic patients with lichen planus and the examination of familial occurrences of lichen planus has suggested that locus HLA-B7 is associated with the condition (173-175).

Leukoplakia

While there is evidence for genetic involvement for certain keratoses, for example white sponge naevus (176,177), leukoplakia appears to be

largely associated with environmental factors (178,179). Aspects of leukoplakia relating to genetic control have been reviewed by Harnden (1984) (180). Evidence indicates that the incidence of leukoplakia may be attributed to variations of oral habits rather than hereditary differences in the populations examined (178,179).

b. Environmental Factors

1. Tobacco

Tobacco can be used in many forms, the most common being inhalation of the burnt products. The tobacco may be air dried or cured before use, these procedures altering the chemical constitution of the leaf and providing the flavour (181). Various substances may be used in conjunction with tobacco in accordance with local customs (vide infra). Conventional smoking of cigarettes is familiar but reverse cigarette smoking which involves the retention of the burning end of the cigarette within the mouth is also practiced in some ethnic groups (182). Bidi smoking involves the use of a type of cigarette made with a temburni leaf to form a conical shape. The length varies between four and eight centimetres and is smoked conventionally. Pipe and chutta smoking consists of the inhalation of smoke produced by burning tobacco in the body of a pipe.

Chewing tobacco retains the leaves within the mouth. Variations adopted by different ethnic groups include the use of the 'pan'. A pan consists of betel leaf, areca nut, lime and tobacco to form a "quid". This is placed within the sulci of the mouth. The use of

snuff, which may be in a dry or moist form, and "Skoal Bandits" which largely contain tobacco similarily require the placement of the manufactured product in the oral sulci.

Lichen Planus

There is little evidence to support an association between tobacco smoking and the prevalence of oral lichen planus except that there does appear to be a significant relationship between plaque lichen planus and tobacco smoke (125,183). Tobacco and betel chewing have been reported to be associated with an increased prevalence of oral lichen planus (97,184). Indeed amongst chewers an "oral lichen planus-like lesion" has been described in the site of placement of the quid (849,185). The question of whether a plaque type lesion of lichen planus could be regarded as leukoplakia arising in oral mucosa affected by lichen planus was raised by Neumann-Jensen et al (1977) (183).

Leukoplakia

Tobacco use has been reported to be associated with leukoplakia since 1851 (5). A significant relationship between tobacco habits and leukoplakia has been reported by numerous investigators (83,146,151,179,183,185-187). Roed-Petersen et al (1972) reported bidi smoking as the strongest aetiological factor in leukoplakia (186). In a ten year Indian study by Gupta et al (1980), it is stated that oral cancer, leukoplakia and erythroplakia occurred almost solely amongst those who practised tobacco habits in one form or other (185).

The association between tobacco use and leukoplakia was further shown by evidence from a study of Bombay policemen in which all cases of leukoplakia were found in the tobacco-using group of which tobacco use was mainly pan chewing with tobacco and the smoking of bidis (151,188). A further study by Gupta (1984) showed that a positive relationship exists between tobacco habits and leukoplakia, a smoking habit having a greater relationship than a chewing habit (189). Confirmation of the aetiological role of tobacco has been shown when leukoplakias have regressed after tobacco use has been stopped (151,185), regression occuring more frequently in tobacco chewers than smokers (151).

The products of tobacco, either burnt or unburnt, have a chemical effect while reverse cigarette smoking has both physical and chemical effects, the combination of heat and burnt tobacco products thus being seen to exert a combined influence on the epithelium of the palate (190). Epithelial hyperplasia is a characteristic feature of snuff induced lesions (191). Palatal nicotinic stomatitis arises in smokers, particularly pipe smokers, the oral lesions presenting clinically as a distinctive array of red spots (minor salivary gland ducts) against the white epithelium. The pathological picture of the epithelium is that of hyperkeratosis with acanthosis. These changes in the oral mucosa may be reversible if the tobacco habit is stopped. Keratotic lesions elsewhere on the oral mucosa caused by tobacco use cannot be clinically distinguished from those caused by other factors. If resolution of the keratosis does not follow cessation of the smoking or chewing habit and elimination of other traumatic factors, the clinical diagnosis of leukoplakia is then generally considered to

be valid.

As previously described, smoking habits have changed markedly in the British population during this century. The Royal College of Physicians in London stated in 1981 that tobacco, in any form, increases the risk of developing neoplasia not only in tissues in direct contact with the products, but also in organs such as the bladder and uterine cervix which are not in direct contact (181). The carcinogenic effects of tobacco may not be apparent for a long time. Increased cigarette tar raises the risk of developing cancer of the lung, mouth and pharynx. The effect of tobacco is further demonstrated in the different cancer incidence between the use of filter and non-filter cigarettes (181,192,193).

2. Galvanism, Corrosion and Allergy related to Dental Restorations

The occurrence of electrogalvanic cells and corrosion is made possible in the mouth by the presence of the aqueous phase, saliva. Dissimilar metal restorations and associated electrical currents were considered in the assessment of aetiological aspects of oral lesions by early authors (13,27,28). Metal restorations have been shown by spectrophotometric methods to produce corrosion products that have been found in the adjacent mucosa (19).

Lichen Planus

Lain (1932) first presented case reports concerning oral electrogalvanic phenomena (25). Lain and Caughran (1933) later

presented observations on an examination of more than 1000 patients having dissimilar metallic restorations (28). In their differential diagnosis of mucosal lesions, seen in association with electrogalvanic injury, lichen planus and lupus erythematosus were included in their list (28). Corrosion of oral restorations and allergic reactions to dental materials are thought to have an aetiological role in lichen planus and the association of gold and amalgam restorations with both leukoplakia and lichen planus have been reported (18,23). Numerous investigators have since reported an association between lichen planus and amalgam restorations (18,194,195). Lichen planus involvement of the buccal mucosa in contact with amalgam fillings in posterior teeth is reported to resolve after restoration with other materials (195).

Patch tests of different restorative metals have been recently reported for patients with oral lichen planus by Eversole and Ringer (1984) (19). While differences were seen between lichen planus patients (21%) and controls (8%) in response to challenge by a panel of amalgam and gold samples, the differences did not reach statistical significance. Eversole and Ringer (1984) considered both their own results and those they reviewed in the literature did not support a major role for metal allergens in the pathogenesis and aetiology of lichen planus (19). Finne et al (1982) discussed the dilemma posed by oral and cutaneous lichen planus in relation to oral restorations and reported that just over half of their 29 patient cohort developed a cutaneous reaction to mercury in a standard test whilst the control population produced only a small positive cutaneous reaction to mercury (3.2%) (195).

Lundstrom (1984) found a mercury reaction with a dermal patch test in twelve of a cohort of 48 patients presenting with lichen planus, clinical signs of amalgam corrosion occurring in 72% of the oral lichen planus patients and in only 28% of the 40 controls (181). While Finne et al (1982) used 0.5% metallic mercury for patch testing (190), Lundstrom (1984) used 0.1% (196). The additional consideration of irritation of the skin rather than hypersensitivity may be of importance as mercury may be toxic, acting as a non reversible competitive respiratory enzyme inhibitor. A recent communication at the British Society for Oral Pathology (1987) examined patch test responses in oral lichen planus patients and controls : no evidence for hypersensitivity to dental restorative metals in these skin tests or in biopsies of oral lichen planus taken adjacent to amalgam fillings was found (197). These reported studies show widely varying prevalences of mercury sensitivity in both patients and controls indicating that further investigations are required.

Leukoplakia

Bloodgood (1932) considered leukoplakia to be occasionally caused by irritation from a tooth "especially one with a metallic filling" (13). Other authors, including Ullmann (1932), considered the electrogalvanic current generated in the oral cavity by dissimilar metal fillings to be important (198,199). Hollander et al (1933) showed the regression of leukoplakia when amalgam restorations were removed (24). Lain's summary (1933) showed 6.2% of the patients examined had leukoplakia in association with the presence of dissimilar metals in the oral cavity (28). More recently Bonoczy

(1979) considered that "electrogalvanic white lesion" may be a useful term to describe a keratosis in association with a definite electrovalent potential (200).

Removal of the dissimilar metals has led to resolution of some leukoplakic lesions (195,198-200). There is evidence that leukoplakia and lichen planus can both arise in mucosa adjacent to metal restorations (190). While the electrogalvanic effects may be important, the allergic reaction to metallic ions may also contribute to the development of the mucosal lesion.

3. Local Irritation

Since the early observations on oral leukoplakia, lichen planus and neoplasia, decayed or jagged teeth and poor oral hygiene have often been said to be associated with the diseases (5,6,7,8,13,15,22,23,25, 26,30). Teeth or prostheses present in the mouth may provide a persistent source of irritation or provide a surface on which products may deposit and affect the adjacent mucosa (13). Partly cured vulcanite dentures were considered by Bloodgood (1932) to form sulphurous products (14). Local irritation may affect oral mucosa in a mechanical, thermal or chemical way, for example burning tobacco exerts both a thermal and a chemical irritant effect that can be seen in nicotinic stomatitis of the palate. Mechanical trauma has long been reported as an important cause of oral lesions (13-15,119,201, 202).

The clinical appearance of oral mucosa in response to friction may be

either an ulcer or epithelial thickening with hyperkeratinization (203). The ulcer is an acute response while chronicity more often produces frictional keratosis (203). The identification and elimination of an irritant related to a white patch and subsequent resolution of the lesion usually confirms the diagnosis of a frictional keratosis. However when there is little change after removal of the putative cause, a diagnosis of idiopathic keratosis generally must be accepted. Sturgis et al (1934) summarised aetiological aspects of oral keratoses and reported that Bloodgood (1932) considered rough teeth and poor oral hygiene to be important in the aetiology of oral cancer (13,195). Mechanical irritation itself is not generally considered to be carcinogenic (202). Historically irritants, including tobacco, chronic periodontitis and carious teeth, were considered to have a small role in the aetiology of oral cancer (204-206). Furthermore there are rare case reports of neoplasia arising in scar tissue (207).

Lichen Planus

There is limited evidence to suggest an association of friction with lichen planus. As with other dermatoses, frictional stimuli may aggravate the lesion and the experimental evidence shows both inter and intra-cellular oedema within the epidermis with friction (208). Damage may also be caused by suction though this may be restricted to the nuclear regions of epidermal keratinocytes (209). Other investigators have examined the effects of trauma to unaffected epidermis of patients with lichen planus and shown induction of eosinophilic bodies in the tissues similar to bodies often found in

lichen planus (210). The lesions in cutaneous lichen planus may sometimes be detected in a linear pattern along a scratch mark or scar (Koebner Phenomenon).

Leukoplakia

The contribution of a frictional component to leukoplakia is evaluated when making the differential diagnosis. Early investigators considered the smoothing down or extraction of rough teeth a useful therapy for oral leukoplakia (13,15,20). In contrast Thoma (1950) did not consider frictional or irritating causes to be aetiological in oral leukoplakia (20). He suggested, in agreement with Hollander (1933), that the occurrence of "leukoplakia" in other sites such as the pelvis of the kidney supported a systemic aetiology (20,23). Cooke (1956) suggested that friction may contribute to leukoplakia as a secondary factor (211). More recently the definition for leukoplakia specifically excludes lesions with a primary frictional cause (73,75).

4. Alcohol

Alcoholic beverages have been ingested by man for thousands of years. Alcohol is taken essentially as an aqueous dilution of ethyl alcohol produced as a by-product of fermentation of sugar by yeast. The fermentation process and distillation of the ferment may concentrate other biologically active products which may be more important than the alcohol in relation to effects on the oral mucosa. The illegal distillation of calvados in France has been reported to provide a high

concentration of carcinogens, though these were not characterised, within the spirit produced (165,204). There is little evidence to implicate ethanol with a direct effect on oral tissues. However the metabolism of ingested ethanol induces cytochrome P-450 and this is reported to activate carcinogens which may be important in the enhanced incidence of cancer in alcoholics (212). A reduction in the thickness of human oral lingual epithelium, primarily in the maturation cell layer due to cell shrinkage, caused by alcohol and tobacco use has been shown by Valentine et al (1985) ; the changes seen histologically were more severe with alcohol (213).

Alcohol has not often been reported as being associated with lichen planus or leukoplakia (68,205). Wilsch (1978) showed that a cohort of leukoplakia patients contained a 1.5 times greater percentage of people using 80 grams or more of alcohol a day (214). This suggests that higher alcohol consumption may be a factor contributing to the aetiology of leukoplakia and Valentine et al (1985) suggested that the functional reductions in the thickness of epithelium may be associated with an increased vulnerability to carcinogens (213,214).

5. Medications

Lichen planus or lichenoid lesions have frequently been reported to be associated with the administration of chemicals and drugs. Bazemore et al (1946) showed that quinacrine used in antimalarial treatment produced oral lesions of lichen planus in almost half the patients receiving therapy (215). Clinical remission on withdrawal of the drug and relapse on subsequent challenge is necessary to demonstrate a

causal relationship with the drug. The clinical and histopathological features of lichenoid lesions and lichen planus are remarkably similar if not identical (203,216). The presence of an eosinophilic or perivascular infiltrate, although variable, is reported to indicate a drug induced lichenoid reaction (216,217). The main categories of chemicals and drugs reportedly associated with lichenoid drug eruptions and lichen planus are illustrated in Table 9.

There is little reported association between drug therapies and the development of leukoplakia (20,23).

TABLE 9

Chemicals and Drugs Associated with Lichenoid Drug Eruptions and Lichen Planus

Category	Example
1. Antimalarials	Chloroquine
2. Methyldopa	
3. Beta blockers	Propanolol
	Labetalol
4. Hypoglycaemics	
5. Diuretics	Frusemide
	Thiazide
6. Non Steroidal Anti-inflammatory Drugs	Ibuprofen
	Diflunisal
7 Photographic chemicals	
8. Penicillamine	
9. Antimicrobials	Tetracycline
	Streptomycin
10. Metals	Gold
11. Salts	Arsenic
	Bismuth

Summarised from Scully and El-Kom (1985) (68).

6. Viral Infection

A viral aetiology for both leukoplakia and lichen planus has been suggested (218,219). In one study HPV type 16 was detected in more than 80% of oral leukoplakias while HPV types 1,2,4,6,11,13,18 were not detected (220). Fry et al (1969) failed to demonstrate viruses or mycoplasmas in lichen planus lesions (221), although more recently HPV types 11 and 16 have been identified in such lesions using DNA hybridization techniques(222).

Papillomavirus DNA has also been found in other keratotic lesions as well as normal oral mucosa (223,224). There is therefore a possibility that pathological changes in oral mucosa may predispose to secondary viral infections and the role of HPV in oral keratoses remains to be substantiated.

Oral hairy leukoplakia seen in patients with Acquired Immunodeficiency Syndrome (AIDS) has been shown to contain Epstein Barr virus (EBV) and Cytomegalovirus (CMV) (225), but the significance of these viruses in an immunocomprmised host is beyond the scope of the present review.

7. Bacterial Infection

Syphilis historically has been implicated with oral leukoplakia (13,18,20,22,24,33). Leukoplakia of the tongue, associated with atrophic glossitis and a positive Wasserman reaction, was reported by Thoma (1941) to occur in up to 27% of oral leukoplakia patients (20). Syphilis is associated with plaque like keratotic lesions commonly

affecting the lip and tongue (20,23). The pathogenesis is unclear although endarteritis obliterans is considered to contribute to the progression of the disease and syphilitic patients show a deficient cell mediated immune response (26). Tertiary syphilis is now a very uncommon disease in Western Europe and serological investigations for syphilis are not now carried out routinely in patients with oral leukoplakia.

Jacobi et al (1933) suggested that a gram negative bacillus may be important in cutaneous lichen planus (226). Other than this report there has been little evidence for a bacteriological aetiology of lichen planus (68).

8. Fungal Infection

The oral cavity provides an environment suitable for the survival of fungi. Candida albicans is a common commensal of the mouth and infects the soft tissues in certain instances (227).

Lichen Planus

In one study yeasts were cultured from swabs of 44% of patients with oral lichen planus (228). Candida albicans accounted for 83% of isolates, other isolates including C. parasilosis and T. glabrata. 37% of the control group of patients also showed positive cultures (228). Histopathological examination of the lesions showed hyphae in three patients, who had either insulin dependent diabetes, Crohn's disease or rheumatoid arthritis. Lundstrom et al (1984) concluded

that Candida hyphae seldom invade the oral tissues affected by lichen planus unless debilitating disease is concurrent (228).

Leukoplakia

Cawson (1966) reported candida hyphae infiltrating the superficial layer of the epithelium in some cases of human leukoplakia (229). Furthermore candida was also reported to occur in speckled varieties of leukoplakia, including erythroplakias (86,166). Shear (1972) regarded the fungus as a secondary invader and its role in the aetiology of leukoplakia remains unclear (86). Evidence from a study on rats with induced oral candidosis implied that the organism may indeed be a co-causative factor in human leukoplakia (230). Earlier work by Roed-Petersen and co workers (1970) described the association of cellular atypia with candidal invasion in 67% of sections but they found no relationship between between the intensity of candida invasion and the cellular atypia in the sections of leukoplakia (231). More recently, the salivary secretor status for blood group antigens has been shown to influence the incidence of infection with Candida albicans in the oral cavity (232). An aetiological role for Candida albicans in candidal leukoplakia has been recently supported, with carcinogenic products being detected in the hyphae invading the epithelium (233). These products have also been shown to affect deeper layers of keratinocytes than the level of hyphae invasion (234).

c. Host Factors

1) Haematological Aspects

Haematological disorders have long been recognised as affecting the oral mucosa. Angular chelitis and atrophic glossitis are associated with iron deficiency anaemia, Vitamin B12 and folate deficiency (235-240). Candidal infection of mucosa may also be associated with iron or folate deficiencies (239). Recurrent aphthous stomatitis has been reported to present with underlying haematological abnormalities which after correction lead to resolution of the disease (241). Atrophy of oral epithelium has been associated with anaemia but it is unclear whether the atrophy preceeds or follows the deficiency.

Evidence that oral keratoses are associated with haematological deficiencies is scant. In a study in which patients with lichen planus, leukoplakia, candidiasis and non specific stomatitis represented 75% of the study population, between two and three per cent were shown to have deficiencies of iron, Vitamin B12 or folate (242). Tyldesley (1983) reported 18.6% of patients with stomatitis to be haematologically abnormal (243). More recently, Challacombe (1986) found that 20.5% of a group of 322 patients with lichen planus, leukoplakia, candidiasis, stomatitis or glossitis had haematological abnormalities (244). This compared with an incidence of 7% in the control population. The number of patients with haematological abnormalities was shown to be significant ($p < 0.05$) for patients with lichen planus (244). Haematological abnormalities were shown to be present in significantly higher numbers in the erosive lichen planus

subgroup than in the non erosive and control groups (243). This may simply be related to those patients with ulcerative lesions in the upper intestinal tract who show iron deficiency anaemia (245). Rennie (1982) has shown an association between iron deficiency anaemia and reduced oral epithelial thickness (246) and this has been supported by more recent studies by Scott et al (1985)(247). Furthermore, the features of epithelial atrophy and high malignant risk seen in Kelly-Paterson syndrome show the potential effects of iron deficiency on the oral mucosa (248).

2) Endocrine Aspects

Evidence implicating an association between an endocrine abnormality and oral keratoses is scant. The reported increased incidence of lichen planus in females is evidence that endocrine status may be an aetiological factor. Early research on treating monkeys with oestrogens showed hyperkeratinization and hyperplasia of the prickle cell layer of the oral epithelium sometimes with an inflammatory infiltrate in the lamina propria being present (249). The incidence of diabetes has been reported to affect patients presenting with lichen planus (68). The aetiological role of diabetes mellitus remains unclear and antidiabetic medications have been shown to produce a clinical and histological picture of lichen planus (see page 56). The relationship between diabetes and lichen planus may not be cause and effect.

3) Psychological Aspects

Stress and the psychological status of patients with oral and cutaneous eruptions have been reported since the nineteenth century. An early association between the nervous condition of patients and lichen planus was made by Wilson in 1866 (38), who also reported cutaneous lichen planus being associated with a spell of good weather (37). Reference to the nervous condition of patients was frequently made by Wilson and other early investigators who recognised the association of lichen planus, both oral and cutaneous, with the level of patient anxiety (41). Andreasen (1968) reported on articles by three earlier twentieth century investigators who had attributed a "nervous or neurogenic theory" and changes in the "vegetative nervous system in the affected areas" as factors in the aetiology of lichen planus (41). This has been supported by case reports of cutaneous lichen planus where the eruption was associated with the "inferior gluteal nerve and popliteal nerve" (50). Severe depression was reported by Pringle (1900) to be associated with the cutaneous eruption of lichen planus (50). Furthermore Waldo (1900) questioned whether the "neurotic sex suffers more than males?" but reported no evidence to support the predeliction of lichen planus for the female (50). Fox (1900) reported a case history of a stockbroker who lost 30,000 pounds sterling and subsequently developed severe lichen planus, endorsing the "neurotic causation of lichen planus" (50).

More recently Lowenthal and Pisanti (1984) examined symptoms in patients with erosive lichen planus who reported more precipitating stressful events than did those patients with other forms of oral

lichen planus (250). Aspects of stress and anxiety relating to oral lichen planus were examined by Allen et al (1986) using Spielberger's State-Trait Anxiety Inventory (STAI) (251). In this study control patients undergoing similar clinical routines, such as biopsy, returned no significant differences to those patients with oral lichen planus (251). It was therefore suggested that oral lichen planus patients have no greater tendency towards anxiety and no more stressful life events than other dental patients (251). In subjects attending the dentist, a degree of anxiety is felt, with 5 to 14% of patients being so anxious that they avoid dental treatment completely (252). This suggests that there remain a group of patients with oral lichen planus with high stress and anxiety who have not attended for dental treatment.

An association between leukoplakia and psychological disease has not been reported.

4) Treatment

The treatment of oral keratoses has varied considerably during the last century but has contributed in a small way to an understanding of their pathogenesis.

Lichen planus

A number of remedies for cutaneous lichen planus were reported during the nineteenth century but there was little information concerning the treatment of oral lichen planus. For cutaneous lichen planus, Wilson

(1866) commonly prescribed "Fowler's solution, three times a day with the use of a lotion of bichloride of mercury, two grains to the ounce, in emulsion of bitter almonds, night and morning; the skin being previously thoroughly washed with the juniper tar soap" (38). It is unclear what Fowler's solution contained. A fero-arsenical mixture to be taken orally, together with mercury bichloride lotion and calcium pentasulphate was endorsed by Wilson (38). Hebra (1868) was reported to advocate arsenic preparations for cutaneous lichen planus (36).

The earliest reported treatment of oral lichen planus was given by Crocker in 1882 (39). The medication consisted of "gentian and magnesia, with thymol locally" (39). Oil of pine was another topical agent considered to be useful (39). However the use of an arsenic adjuvant was encouraged when the patient could tolerate that treatment (39). Fox (1883) was reported to use "inunction of olive oil and mineral acids internally" (39).

Little (1919) presented a paper related largely to cutaneous lichen planus where the treatment comprised an intramuscular injection of a combination of arsenic and mercury every two days for six weeks (59). Other physicians also recommended the treatment of cutaneous lichen planus with mercury (253). This medication proved useful in reducing the itch and controlled acute and chronic eruptions (59,253). Interestingly, dietary restriction was recommended by Bulkley (1919), with a diet of bread, butter, rice and water recommended at the onset of the disease (52). X-rays proved disappointing but cryofreezing of hypertrophic lesions was reported as a more satisfactory form of treatment of skin lesions (59).

Bismuth injections, soothing ointments, ultraviolet light, vitamins, sedatives and X-rays were reported treatments for cutaneous lichen planus carried out in the University of Chicago between 1942 and 1952 (63). In addition, Tompkins (1952) noted that reassurance was valuable in the management of the patients (63). Recurrences were 12% of the 41 patients in the study and it was noted that mucous membrane involvement tended to prolong the duration of the disease (63). Thoma (1950) continued to recommend arsenic and mercuric preparations in the treatment of oral lichen planus (20). In Britain, Cooke (1958) reported the use of 2% aureomycin mouth washes for oral lichen planus (64). The smoothing of rough teeth and dentures was also reported as valuable (64). Ulcers in lichen planus were painted with one per cent aqueous gentian violet to prevent secondary infection (64). Cooke (1958) also suggested that treatments for cutaneous lichen planus were valueless in the treatment of oral lichen planus (64). Shklar (1972) endorsed the more resistant nature of oral lichen planus to treatment, compared to cutaneous lichen planus (65).

By 1970 corticosteroid preparations were widely available and Shklar (1972) restricted their use in either topical or systemic form to erosive lesions of oral lichen planus (65). Sleeper (1967) described the use of intralesional and sublesional injections of Triamcinalone acetamide for oral lichen planus with success (254). Betamethasone was also reported to be a successful medication for oral lichen planus (255). A wide range of steroid preparations for topical, intralesional and systemic use now exist. Topical and intralesional steroids are useful for localised areas of oral lichen planus and are administered to the lesion in the form of gels, creams, aerosols or

lozenges or into the connective tissues beneath the lesion by means of injection. The use of systemic steroids is generally restricted to those cases of more severe lichen planus where erosion and atrophy involve oral or genital sites, the administration of steroid tablets being more common in outpatient clinics. Steroids generally available are illustrated in Table 10 (68,256).

The possible mechanisms of action of corticosteroids are described by Pedersen and Klausen (1984) (257). Corticosteroids inhibit many of the processes associated with inflamation when administered in supra-physiological concentrations (257). These include the stabilisation of cell membranes and thus a reduction of prostaglandin and lysosomal release in the inflamed tissues (257). In lichen planus and other inflammatory conditions, steroids may exert an anti-inflammatory effect on lesional and circulating lymphocytes, thus modifying the level of tissue inflammation. Lichen planus is more often than not controlled by the administration of steroids although cure is infrequent for this chronic condition.

Other treatments used include the administration of griseofulvin and levamisole (258,259). Although griseofulvin is concentrated in keratin, the action of this antifungal medication on lichen planus is unclear. There are also reported side effects from these medications including the appearance of lichenoid eruptions with the use of levamisole which is an immuno-suppressant agent (259). Antibiotic treatment of cutaneous lichen planus has been attempted, but Samman (1954) abandoned a trial of chloromycin, aureomycin and terramycin when no beneficial effect was seen (70).

Table 10

Medications Available for the Treatment of Lichen Planus.

Systemic	(1) Corticosteroids
	-cortisone
	-methylprednisolone
	(2) Female Hormone Replacement
	(3) Adrenocorticotrophic Hormones
Topical	Corticosteroids
	-hydrocortisone
	-triamcinalone
	-betamethasone
	-prednisolone
	-flurandrenolone
	-fluocinonide
Intralesional	Corticosteroids
	-hydrocortisone
	-dexamethasone sodium phosphate
	-triamcinalone acetonide
	-methylprednisolone

The use of sedatives and tranquillizers has been unhelpful in the management of lichen planus (63) however psychotherapy has been reported to have some value (260,261). Thoma (1950) reported the use of mercury and arsenic preparations and valued "..hygiene, good food, sunshine and freedom from worry." in the treatment of lichen planus (20). More recently the periodontal and prosthetic treatment of patients with oral lichen planus has been evaluated and it was found that gingival involvement may be improved by optimum plaque control and periodontal treatment (262). The use of chlorhexidine in a 0.2% aqueous solution as a mouthwash has recently been suggested to be remarkably beneficial in the control of oral lichen planus (Holmstrup P. personal communication).

Leukoplakia

A variety of treatments for oral keratosis were in use during the nineteenth century most of which related to the elimination of possible factors causing local irritation (page 53). Bloodgood (1932) even advised dental clearance to eliminate local factors (13). Otherwise local excision of the lesion using surgery, cryotherapy or laser treatment has been advocated. The main reason for such treatment is the prevention of malignant change and there is no record in the literature of steroid therapy being recommended for the management of leukoplakia.

Historically small lip keratoses were treated with single radium applications while larger lesions could receive various radium therapies and, in certain cases, high X-ray dosing (24). If the

removal of local irritants did not resolve small oral leukoplakias after three months then removal effected by electro-dessication under local anaesthesia was often instigated (27,30,54).

Symtomatic treatment of leukoplakia with weak alkaline mouthwashes such as sodium bicarbonate and the use of chromic acid application for ulcerated areas was recommended by Fitzwilliams (1927) (26). Discomfort, especially of the tongue, indicated application of lanoline type creams with topical anaesthetic agents (26). Systemic associations, including syphilis and Vitamin A deficiency, were investigated when local irritant causes had been eliminated and the lesions persisted (20,22). The concern for malignant transformation persisted and observation of the leukoplakia becoming thickened or ulcerated indicated excision of the lesion at the earliest opportunity (27,30).

Thoma's classification of leukoplakia, described earlier, included four types or grades which varied in clinical appearance (20). The fourth type was a "neoplastic leukoplakia" which presented in a verrucose form and had a recognised neoplastic potential (20). Treatments directed at these specific types of leukoplakia varied and were dependent on histopathological features (20). Interestingly, the inflammatory infiltrate in the subepithelial tissues was a common picture with all the grades of leukoplakia, however the variation of keratinization was seen to help to distinguish these gradings (20). The "neoplastic leukoplakia" was probably early carcinoma invading the deeper tissues and the treatment by excision and endothermy was the only appropriate course of action (20,26).

Today there are three main ways in which leukoplakia is treated, the choice being determined by the clinicians' view as to whether or not the lesion has a local cause and evaluation of its malignant potential, the latter being influenced by the site of the lesion and the degree of epithelial dysplasia.

1. Management limited to removal of local irritants including tobacco, either in smoke or leaf form and the regular monitoring of the lesions (81,82). As there is definite risk of malignant transformation that remains unpredictable, the maintenance of regular review is essential (74,263).

2. Medical management for candidal leukoplakia involving antifungal therapies (119). A variety of other therapies for leukoplakia have been suggested, including topical or systemic treatment with Vitamin A and fibroblast interferon (54,264,265).

3. Surgical removal by means of cryotherapy or surgery of the leukoplakia (20,119,120,264,265).

MICROSCOPIC FEATURES OF ORAL KERATOSES

Before describing the microscopic features of oral keratoses, a short account is given of the structure of normal oral epithelium.

a. Normal Oral Epithelium

Human oral mucosa is derived from ectoderm. It is a moist surface tissue and has many characteristics of skin. Oral mucosa provides a continuous covering of stratified squamous epithelium, functionally divided into three types (266).

1. A covering mucosa, involving buccal and labial surfaces, ventral surface of tongue, floor of mouth and soft palate, which is normally non-keratinized.
2. A masticatory mucosa, restricted to gingivae and hard palate, which is normally keratinized.
3. Specialised mucosa is seen on the dorsum of the tongue where gustatory buds and papillae are present.

The supporting connective tissue is adapted to the functional requirements of the mucosa. Masticatory mucosa is adherent to underlying bone by dense fibrous tissue. Covering mucosa is attached by less dense connective tissue to the underlying muscle (266). Connective tissue papillae interdigitate with the epithelium and vary in both density and height according to site (267,268). The metabolic supply for the epithelium is provided by the vessels permeating the connective tissue papillae (268,269). The epithelium consists

predominantly of keratinocytes but there is an estimated 10% component of non-keratinocyte cells consisting predominantly of Langerhans cells and melanocytes (270). In inflamed tissues the percentage of non-keratinocytes and inflammatory cells may increase (271). External physical stimuli such as chemical agents and friction and the presence of subepithelial inflammation may affect the turnover rate of the epithelium (271-273).

1. Keratinocytes

The site of cell proliferation in the epithelium is the germinal cells that exist adjacent to and within three cell layers of the basement membrane (274). Additionally cell division may be clustered (266,274). Accounting for the greatest proportion of cells in the oral epithelium, keratinocytes divide at the basal and parabasal layers, mature and progress to the surface. Non-keratinized epithelium matures with the production of tonofilaments that are in a loose network (275,276). The cells become increasingly stratified towards the surface. Keratohyalin granules and glycogen may be present in the superficial part of the epithelium (276,277). In keratinised epithelium maturation of keratinocytes in the prickle cell layer produces tonofilaments which are visible microscopically (276). At the granular layer, keratohyalin granules dominate and associate with the tonofilaments to form the sulphurous matrix of microfibrils at the keratinized surface (276,278). The keratinized surface is a microfibrillar mass. In orthokeratosis, the keratinocytes lose all visible signs of organelles except for the filaments and in parakeratosis, pyknotic nuclei remain within the keratin matrix (277).

The keratin surface is tough and insoluble in normal oral conditions but this may be altered (279).

Keratinocytes produce a family of proteins called keratins (275), different types of epithelium producing different types of keratin. The keratins comprise 19 polypeptides that can be divided into two groups, I and II, according to immunoreactivity, isoelectric focusing and DNA hybridisation (275). Keratins are gene products and are produced in different quantities according to the type of epithelial keratinization (275). There is no evidence to suggest that different types of keratin are produced in oral keratoses compared to normal oral mucosa and they have not been investigated in this study.

Keratinocyte cell surface carbohydrates provide a wide range of epitopes including the blood group antigens. The significance of the distribution of blood group antigens in keratiocytes is discussed on page 78.

2. Langerhans cells

These are a dendritic type of cell seen in the epithelium, the cell being part of a family of cells often termed dendritic leukocytes. Langerhans cells are thought to be continuously seeded out of the bone marrow to the epidermis where mitotic activity may also occur. Their role in both normal and pathological tissue is not fully determined but primary antigen processing is a major function (280-283).

3. Melanocytes

Melanocytes are pigment (melanin) producing cells that are found in the basal layer of epithelium. Embryologically, melanocytes are derived from the neural crest. Melanin is conveyed to the keratinocytes as melanosomes by dendritic processes (284). Melanin granules are visible under light microscopy in melanocytes and keratinocytes, and may also be seen in melanophages which are macrophages containing melanin seen in the upper parts of the connective tissue (285).

b. Lichen Planus

The histopathological features of oral lichen planus were first reported by Dubreuilh in 1906 (47). It was clear that histological features of oral lichen planus are similar to those of cutaneous lichen planus (47). The following histopathological features are commonly described in oral lichen planus (47,48,59,61,62,64,65,286-299).

1. Epithelial Features

1. Generally hyperplastic or acanthotic but not infrequently is atrophic
2. Hyperkeratosis- either parakeratin or orthokeratin
3. Increased intercellular spaces in basal epithelium (liquifactive degeneration) (287,288).
4. Presence of colloid bodies which are thought to

represent apoptosis (287,290).

5. Pointed rete ridges (saw tooth) (65).
6. Prominent prickle cell layers (47,66).
7. Presence of a granular cell layer (290).
8. Mononuclear cell inflammatory infiltrate in the basal layers of the epithelium (65).

2. Epithelial/Connective tissue interface

1. Basement membrane zone damage (291).
2. Thickening of basement membrane with fibrin or immunoglobulin (292-294).
3. Disruption of basement lamina (292).
4. Oedema and splitting away of epithelium from connective tissue (295).

3. Connective tissue

1. Apoptotic bodies (as within the epithelium) (296-299).
2. Presence of colloid bodies which are thought to represent apoptosis (287,289).
3. Dense band of mononuclear cells in the upper lamina propria, almost entirely composed of lymphocytes and with plasma cells inconspicuous or absent.
4. Distinct delineation between mononuclear cell infiltrate in the upper lamina propria and deeper connective tissue (47).

No single histological feature is diagnostic for lichen planus. In practice a pathologist makes a subjective diagnosis based on a study of the features described above. Similar microscopic features are seen in lichenoid drug reactions when the diagnosis must be related to the appearance of clinical lesions upon challenge by the respective drug or drugs, and re-introduction of the drug after cessation of the therapy should then re-establish the clinical condition.

The earliest changes, illustrated by electron microscopy, in lichen planus appear to be within the epithelium (297). Injury of the basal cells leads to damage and the development of intercellular spaces (280). The hemidesmosomes between basal epithelial cells and the basement membrane appear to deteriorate, leading to splitting of the epithelium from the basement membrane (288). Disruption of the basal lamina is thought to be followed by attempts at repair, perhaps leading to loss and irregular thickening of the basal lamina (292). Ultrastructural studies indicate that colloid or Civatte bodies retain a filamentous structure consistent with the epithelial or keratinocyte origin of these masses (287,300,301). Since these epithelial changes may be seen in other inflammatory conditions, their significance for lichen planus remains unclear (300). Blood group substances are lost in the prickle cell layer (302) and the basement membrane shows antigen loss by reduced carbohydrate binding with Concanavalin A (ConA) and Ricinus communis agglutinin (291,293). Vascular dilation, oedema and disruption of the basement membrane zone occur (303-305). More recently the use of monoclonal antibodies to identify epitopes present in lichen planus has helped in the understanding of pathogenic processes and this will be described in the next section.

c. Leukoplakia

The histological features of leukoplakia are not specific and reflect varying degrees of keratosis, epithelial thickness, epithelial dysplasia and chronic inflammatory cell infiltrate (164,306). The keratin may vary in thickness, be ortho or parakeratin, or a mixture of both. The lamina propria may be irregularly infiltrated by mononuclear cells, predominantly lymphocytes and plasma cells (20,81,82). The important histopathological feature of idiopathic keratosis regarding behaviour is thought to be the degree of dysplasia of the epithelium (82,83,203,307). Although this has been questioned, it would appear that there are no better indicators at present (161).

Objectivity in histological assesment remains a major problem and attempts to standardise this evaluation have been made (141,161,307-309). A system using photographic standards for the evaluation of epithelial dysplasia was put forward by Smith and Pindborg (1969) (141). This was followed by work from Kramer and co-workers (1970) which used computer aided analyses for the evaluation of histopathological features of oral keratoses including lichen planus (153,154). Franklin et al (1980) reported stereological methods for assessment of epithelial dysplasia (309). Distinction between dysplastic and normal epithelium was made using stereological examination of nuclear/cytoplasmic ratios and nuclear density (309). The following histopathological features of epithelial dysplasia are commonly described in oral idiopathic keratoses (145,146)

Gross Structure

a. irregular epithelial stratification

b. drop-shaped rete processes

c. hyperplasia of basal cell layer; cell crowding creating appearance of multiple layer of basal cells

d. keratinization in prickle cell layer (disturbed maturational sequence)

e. reduced intercellular coherence

f. disturbed polarity of basal and or supra basal cells

Cellular features

g. increased mitoses and abnormal mitoses within the thickness of the epithelium

h. pleomorphism

i. hyperchromatism

j. increased nuclear/cytoplasmic ratio

k. prominent nucleoli

Since 1950 the infiltrate in idiopathic keratoses has been largely overlooked in the histological assessment. Shafer and Waldron (1961), while recognising that an infiltrate was present in the underlying connective tissue, stated that "the presence of inflammation in leukoplakia has been noted (54% of biopsies). However, the significance of this finding is not understood and possibly may not be related to the lesion per se..." (81). Later Shafer and Waldron (1975) disregarded the inflammatory infiltrate of the lesions examined, concentrating on the dysplastic features of the epithelium (82).

d. Histopathology of Discoid Lupus Erythematosus

Lupus erythematosus is generally regarded as an autoimmune process involving both humoral and cell mediated immunity, in which a large number of autoantibodies directed against nuclear and cytoplasmic components have been identified. In the discoid type of the disease (DLE), clinical lesions are confined to skin and mucosal surfaces. On the oral mucosa, erythematous plaques or erosions associated with white striae radiating from the periphery of the lesion are seen. In the systemic type (SLE), skin and mucosal lesions are relatively mild and the disease is dominated by multiple systemic involvement affecting joints, kidneys, heart and lungs. The histopathological diagnosis of discoid lupus erythematosus is based on the presence of the following major features (310,311).

1. Pseudoepitheliomatous hyperplasia
2. Hyperorthokeratosis with vertical clefts in the surface plugged with keratin
3. Liquifactive degeneration of basal epithelial cells
4. Deep inflammatory infiltrates (diffuse, perivascular or focal aggregates)
5. Thickening of the basement membrane zone.

There are further, less important, histological features presenting in discoid lupus erythematosus. These include the presence of colloid or Civatte bodies, an eosinophilic cell free zone separating epithelium from the inflammatory infiltrate and the predominance of a mononuclear cell infiltrate with occasional plasma cells (310,311). Pathologists

value the distribution of lymphoid aggregates in the differential diagnosis of oral discoid lupus erythematosus. Pindborg (1980) included discoid lupus erythematosus in his list of precancerous conditions but Schiodt (1984) noted that the literature had not documented intraoral carcinoma arising in pre-existing lesions of discoid lupus erythematosis (141,311).

e. Histopathology of Frictional Keratosis

It is a commonplace observation that chronic friction to the skin leads to thickening and the formation of epidermal callus. The only proper study of the effects of friction on oral mucosa was that described by Mackenzie and Miles (1973) who subjected hamster cheek pouches to controlled frictional stimuli (312). Friction for periods up to 14 days produced thickening of the keratin, an increase in the size and number of prickle cells, and increased mitotic activity

f. Histopathology of Smoking Keratosis

While the smoking of tobacco is widely regarded as an important cause of oral keratoses, there is little specific information in the literature concerning histological changes directly attributable to tobacco smoke. Experimental animal studies have not been helpful. An exception to this is the lesion of the palatal mucosa frequently seen in persons who are heavy pipe smokers. Nicotinic stomatitis of the palate shows epithelial thickening and hyperkeratinization with the ducts of the mucous glands dilated and the orifices partially occluded by keratin. Pindborg et al (1980) described a chevron like

keratinization in a number of tobacco smoke induced lesions of the oral mucosa, with vertical streaks of parakeratin in the upper layers of the epithelium (313). The epithelial cells in between the vertical streaks of parakeratin sometimes have enlarged vacuolated cytoplasm. Smokeless tobacco directly applied to the buccal mucosa produces a parakeratinized surface with epithelial thickening (or occasionally atrophy), often with vacuolisation of prickle cells. Stromal inflammation is variable and epithelial dysplasia is unusual (149). In summary therefore, the effects on the oral mucosa of smoking tobacco are likely to be hyperkeratinization or parakeratinization (if the epithelium was previously non-keratinized), epithelial thickening (or occasional atrophy) and variable stromal inflamation.

g. Histopathology of Hereditary Keratoses

Several hereditary keratotic disorders of the oral mucosa are characterised by disorders of keratinization and share a common clinical finding of a mucosal white lesion. White sponge naevus is a autosomal dominant transmitted condition that may be mistaken clinically for leukoplakia. It presents as a deeply folded white lesion affecting several mucosal surfaces, the lesions being thick with a spongy consistency and showing bilateral symmetry. The buccal mucosa is generally involved. Microscopically the epithelium is acanthotic and parakeratinized . The prickle cells show marked hydropic or clear cell change with nuclei somewhat pyknotic and often eccentric in location.

IMMUNOLOGY OF ORAL KERATOSES

Introduction

During the latter half of this century significant advances in understanding immune mechanisms and disease have been made. Early experiments, largely using micro-organisms, showed basic immune responses but more recently it has been realised that the gut and skin associated immune mechanisms have major controlling functions. It is still unclear how oral mucosal disease is influenced by such immune systems and further studies are therefore required. The humoral and cellular immune aspects of lichen planus and leukoplakia will now be reviewed.

a. Humoral Immunity in Lichen Planus

1. Immunoglobulins

Shuttleworth et al (1986) reviewed the literature pertaining to immunoglobulins and autoimmunity in studies on lichen planus (314). Some previous studies had suggested that systemic immune mechanisms are important in the pathogenesis of lichen planus (315,316). However this appears to be an inconsistent feature if serum immunoglobulins are evaluated as markers of immunological abnormality. Table 11 summarises values reported by nine investigators. While the similarity of clinical features in lichen planus and graft versus host disease may be suggestive of a common aetiology, this does not appear to be reflected in humoral immune profiles of lichen planus (317).

a) Serum Immunoglobulin G. Lundstrom (1985) found a higher level of serum IgG in patients with lichen planus than in a control group (318). An important feature of this patient group was that the majority, twenty eight out of thirty four, had atrophic-erosive type lichen planus (318). This association of raised IgG levels with lichen planus has also been reported by Schroeder (1981) who noted raised levels of IgG in erosive lichen planus and mucocutaneous lichen planus (319). While Griffiths et al (1974) did not show differences in mean levels of serum immunoglobulin values between patients and controls, elevated levels of IgG were present in 44% of patients with erosive lichen planus and this was attributed to secondary infection (320). Sklavounou et al (1983) were able to show raised serum IgG levels for both ulcerative and reticular lichen planus compared to controls (321).

b) Serum Immunoglobulin A. Serum IgA normally accounts for approximately one fifth of the serum immunoglobulins (322). Reduced levels of serum IgA in lichen planus were reported by Stankler (1975) and Sklavounou et al (1983) (321,323). However Mayhood (1981) reported raised levels of serum IgA for resolved cutaneous lichen planus when compared to previous active lichen planus (324). Other investigators have failed to show any change in the serum IgA level in lichen planus (314,318,319,325-327).

c) Serum Immunoglobulin M. The pentameric IgM molecule is generally restricted to the vasculature. Being a strong binder of complement, IgM can activate the complement reaction (322). IgM is raised in systemic lupus erythematosus, rheumatoid arthritis and may have a role

in other chronic inflammatory conditions (311).

Stankler (1975) and Jacyk and Greenwood (1978) recorded decreased levels of IgM in cutaneous lichen planus (323,326), while Mahood (1981) reported raised levels of IgM in patients after resolution of lichen planus (324). Other investigators have found no major alteration of serum IgM in lichen planus (see Table 11). Furthermore patients with oral erosive lichen planus have not been shown to have any alteration of serum IgM (320,321).

TABLE 11

Serum Immunoglobulins in Lichen Planus

		IgG	IgA	IgM	No. of Patients	Reference
1969	Lehner	Dec	-	N	20	(325)
1974	Griffith et al	N	-	-	35	(320)
1975	Stankler	N	Dec	Dec	13	(323)
1978	Jacyk & Greenwood	N	N	Dec	42	(326)
1981	Mahood	Inc	Inc	Inc	31	(324)
1982	Scully	N	N	N	35	(327)
1983	Sklavounou	Inc	Dec	N	50	(321)
1985	Lundstrom	Inc	N	N	34	(318)
1986	Shuttleworth	N	N	N	54	(314)

Serum Immunoglobulins in Erosive Lichen Planus

1974	Griffith et al	Inc	Inc	N	35	(320)
1981	Schroeder	Inc	N	N	-	(319)
1983	Sklavounou	Inc	Dec	N	13	(321)

Adapted from Shuttleworth et al (314)

(Dec = Decreased, N = Normal, Inc = Increased)

2. Complement

The series of serum proteins known as complement provides a variety of biological mechanisms that may amplify humoral and cellular immune functions. The C3 component has been shown to be present in normal values in the serum of lichen planus patients (320,321). Complement is present at the basement membrane zone in the oral lesions of lichen planus (311,328-330). Additionally complement is seen to be present frequently in other oral inflammatory conditions including leukoplakia and discoid lupus erythematosus (329). The presence of C3 does not appear to contribute to the differential diagnosis of these white patches (311,329).

3. Autoantibodies

Lichen planus has been suspected of being an autoimmune disorder (314,330). Shuttleworth et al (1986) assessed fifty four patients with lichen planus for autoimmune disorders and failed to show any increased prevalence in the patient group over the control group (314). Lundstrom (1985) also failed to find any significant evidence for autoimmunity (318), despite 27% of oral lichen planus patients, compared to 9% of controls, showing positive titres of rheumatoid factor (RF) and antibodies to antinuclear factor (ANF) (318). Schiodt et al (1982) failed to show significant anti-DNA antibodies in lichen planus patients (330). Furthermore the presence of markedly raised anti-DNA antibodies was found in 4% of patients with discoid lupus erythematosus, lichen planus and leukoplakia and this parameter had no differential diagnostic value (311,330). Lichen planus has also been

associated with late onset hypogammaglobulinaemia, thymoma and alopecia areata but the relationship between these conditions and lichen planus remains unclear (331,332).

b. Humoral Immunity in Oral Leukoplakia

The assessment of humoral immunity in patients with oral leukoplakia, including serum levels of immunoglobulins has not been reported in the literature. Immune changes in oral cancer may be the progression of those identified in leukoplakia and so may be relevant to leukoplakia.

For oral cancer the main classes of immunoglobulin have been assessed with a view to prognostic indicators (333-336). Serum IgG, IgM and IgD levels appear to be unaltered in patients with oral cancer (334,335).

Parotid saliva has also been reported to be unaltered in oral cancer patients compared to controls (334,335). Salivary IgA in oropharyngeal cancer has been reported to be raised but this was considered to be an association with tobacco and alcohol use and not directly related to the assessment of oropharyngeal malignancy (337). Patients with primary oral and pharyngeal cancer demonstrated a two fold increase in IgA in whole saliva over a control group (336). It can be anticipated that the oral or pharyngeal sites of ulceration may have contributed to the raised levels of IgA in the whole saliva through exudate of tissue fluids from these carcinomas. It is therefore not surprising that patients with recurrences of oral or pharyngeal carcinoma showed the greatest elevations of salivary IgA

(346). Other studies however do not confirm these results and do not indicate that any major differences in salivary IgA levels exist between oral neoplasia and healthy controls (338).

Reviews of immunology of oral cancer do not attribute humoral immune defects to the acquisition or progression of cancer (159,165,339,340). The assessment of serum complement and autoantibodies has not been reported for idiopathic keratoses.

c. Cellular Immunity in Oral Lichen Planus and Leukoplakia

Limited (in vitro) studies of cellular immunity in lichen planus and idiopathic keratosis have been reported and understanding at present is dependent largely on the morphological information obtainable from the immune infiltrates of these lesions (69,165,204). The presence of immunocytes in the epithelium or lamina propria is indicative of immune mechanisms in operation.

Lichen Planus

The cell infiltrates in oral and cutaneous lichen planus have been identified using various techniques including sheep red cell tests, enzyme histochemistry and monoclonal antibody studies. The presence of predominantly T lymphocytes of either suppressor or helper phenotypes has been shown (341,342). Suppressor lymphocytes have been shown both in the lamina propria (in large numbers) and within the epithelium (342,343). Macrophages have also been demonstrated in oral lichen planus using immunoperoxidase techniques and monoclonal

antibodies (344-346). Langerhans cells have been shown to be present in the epithelial para-basal cell layer and juxta-epithelial tissues (341,345,347).

The presence of HLA-DR epitopes on epithelial cells in oral lichen planus is considered to be part of keratinocyte antigen processing (345,348,349). Langerhans cells have also been shown to express surface and cytoplasmic HLA-DR sites (345,349). This expression has been shown in other mucosal diseases, notably oral candidosis, gingivitis and graft versus host disease (350,351). The distribution and number of T lymphocytes are reported to be directly related to the epithelial expression of HLA-DR epitopes (350).

B lymphocytes and plasma cells are an infrequent finding in cutaneous and oral lichen planus (343,352). There has however been recent evidence suggesting that some B cells do exist in close proximity to helper T cells in the infiltrates seen in lichen planus (349).

An unidentified substance, designated lichen planus specific antigen (LPSA), has been demonstrated in the lesions of cutaneous lichen planus but not in oral lichen planus lesions (353,354). This antigen is present in the granular and upper prickle cell layers (353). Incubation of serum from four patients having oral lichen planus with allogeneic cutaneous lichen planus lesional sections showed positive LPSA in two of the four patients (354).

Deposits of IgG, IgA, IgM and fibrin have been reported in lichen planus in the basement membrane zone (352,355). A particular

epidermal protein, involucrin, has also been shown in oral lichen planus (356). The importance of these immunoglobulin deposits and involucrin is unclear.

Obtaining cells for (in vitro) assessment of the immune infiltrates of tissues is difficult. Lichen planus has a dense and distinctive immune infiltrate and this has not been fully examined with such techniques (357). Preliminary investigations by Sarkony and Gaylarde (1972) showed lymphocyte transformation of autologous lymphocytes when cultured with a homogenate of the dermal lesion (358). The dermal infiltrate appeared to be proliferating lymphoblasts (359).

Leukoplakia

Phenotypic characterisation of immunocytes present in leukoplakia has not until recently been possible (350). As with studies on lichen planus, the advent of monoclonal antibodies has been useful in identifying the phenotypes of the infiltrates. Migliorati et al (1986) when studying sections from twenty one biopsies of red or white oral lesions (6 hyperkeratosis, 3 mild dysplasia, 4 severe dysplasia and 8 squamous cell carcinoma) found a predominance of T lymphocytes on immunostaining (360). Helper (T4) cells were present in higher numbers than suppressor (T8) lymphocytes (360). Differences were reported between mild and severely dysplastic lesions for the T4/T8 ratio and the mononuclear cell infiltrate contained low numbers of B cells (B1) while in the hyperkeratotic and mild dysplastic tissues examined B cells comprised 37% of the total and in the severe dysplastic and squamous cell carcinoma tissues comprised 58% (360).

Monocytes and null cells were not found when using the OKM1 monoclonal antibody (360). Activated T lymphocytes, Langherhans cells, monocytes and B cells distinguished by the appearance of HLA-D/DR and Ia like antigens, produced intense reactions (360). Langherhans cells were noted in epithelial cell layers (360). Migliorati et al (1986) further showed a low proportion of natural killer and killer type cells distinguished by a leu-7 monoclonal antibody (360). These cells were invariably seen in the connective tissues.

Interestingly, follow up of one patient over a year showed an increase of helper cells (T4) in the lesion from the original hyperkeratosis to mild dysplasia. The suppressor cell T8 population remained relatively constant, the increase in T4 cells accounting for a raised T4/T8 cell ratio (360).

The infiltrate associated with carcinoma, when pronounced, has been associated with improved prognosis for the patient (361) but this simplistic concept has been questioned as understanding of carcinogenesis and immunology has developed (362). Woodruff (1978) considered reduced immunity to facilitate the emergence of altered or neoplastic cells (207). Den Otter (1986) reviewed the concept of immune surveillance and natural resistance and suggested three points:

"1. Tumour cells do not arise frequently
2. Tumour cells may be antigenic or not
3. There is no need to postulate a very strong immune surveillance or natural resistance system." (362).

This implies that immune responses in malignancy may vary significantly between individual patients.

1. Lymphocyte Transformation

Lehner (1970) studied lymphocyte transformation of peripheral blood lymphocytes against homogenates of leukoplakia tissue (363). Although this represented a mixed lymphocyte response of T and B cells, transformation appeared to become increasingly impaired with the severity of the epithelial dysplasia. The homogenate, while probably containing the epithelial antigens against which the lymphoblasts are reacting, must have also included numerous other proteins that can initiate blastic transformation of lymphocytes. Lehner suggested that depression in "cell mediated" immune responses may accompany the onset of carcinoma (363).

2. Migration Inhibition

Both T and B lymphocytes can produce migration inhibition factors. The method for demonstrating migration inhibition relies on immunologically active cells inhibiting the migration of macrophages (364). Roed Petersen et al (1973), using soluble extracts of leukoplakia tissue and homologous oral mucosa, investigated migration inhibition and tested eleven patients with leukoplakia (365). Of these patients, five showed inhibition of migration. However the assessment of cell mediated activity against the epithelial tissue of leukoplakia has to be viewed in light of toxic effects as well as specific immune effects (365). The function of peripheral blood

lymphocytes may also be different from the function of the lesional cells present. Indeed the lesional cells are more likely to be of a specific population that associate with the lesional site due to chemotactic information (366).

3. Cytotoxicity Assays

While extensive work has been done in relation to neoplasia and cytotoxic effects of immunocytes, little has been reported for oral keratoses. There are problems obtaining appropriate controls for patient lymphocytes and the effects of killer and natural killer cells within the test sample make the interpretation of results difficult. Indeed there is evidence that virally induced tumour cells are killed by cytotoxic T lymphocytes and natural killer cells and the immune activity against other tumour cells is weak (362,367).

4. Anti-Viral Immunity

While the Herpes simplex viruses types 1 and 2 has been implicated in both oral cancer and leukoplakia, papilloma virus antigens have also been detected in the lesions (368,369). This implies that the infiltrate seen in a leukoplakia may be a response of viral specific cytotoxic T lymphocytes. This is confirmed by results obtained by Becker et al (1985) which showed suppressor/cytotoxic T cells more common at the basement membrane area of leukoplakias (370,371). Qualitative and quantitative immunocyte differences appear to exist between lichen planus, leukoplakia and carcinoma in both the epithelial and the connective tissue compartment suggesting they do

not contain a common virus (371,372). Penetration pathways for different compounds through oral epithelia have been recently described by Squier and Leisch (1988) (373) and permeability characteristics of oral epithelium may have an important bearing on the aetiology of oral keratoses.

7. Study Aims and Objectives.

From the literature review it appears that the diagnosis and management of oral keratoses is generally staight forward, but destinguishing different types of keratoses eg. lichen planus, may be more difficult. Many reports restrict assessment to pre-selected groups of oral keratosis patients and avoid those cases where difficulties in diagnosis and management are perceived to complicate the study.

The aim of this thesis was to assess clinical, pathological and immunological aspects of oral keratoses using a new system of classification sensitive to the presence of an underlying population of infiltrating immunocytes. It was also necessary to retain the conventional diagnostic groups so comparisons could be made with other studies.

The present thesis is based on a personal clinical and laboratory study of 159 patients presenting in a dental hospital with oral keratoses over a three year period.

The objectives of the present investigation therefore were to provide answers to the following questions.

1. Are the following investigations of value in the distinction between the different keratoses?

   a. Haematological parameters

   b. Immunological parameters

   c. Drug history

   d. Systemic Disease

2. Does histological examination always clearly distinguish between oral keratoses and does the categorisation of oral keratoses into infiltrated and non infiltrated groups provide a useful basis for clinical management?

3. Are certain general or systemic factors such as hypersensitivity to substances used in dentistry associated with different types of oral keratoses?

4. Are smoking and alcohol habits linked to particular oral keratoses?

5. Is the local infiltrate associated with the keratotic lesion destinct from peripheral blood lymphocytes when assessed using 'in vitro' cell cultures and are there quantifiable differences between patients and controls for peripheral blood lymphocyte transformation?

The hypothesis that the cellular infiltrate, present or absent from the oral keratosis, is important in determining the malignant potential of the lesion was formulated at the beginning of the study. There was no evidence in the literature that the cellular infiltrate had been assessed for patients attending a clinic with a range of oral keratoses. This aspect of the lesional infiltrate has been largely overlooked in favour of the features of epithelial dysplasia during the clinical management of oral keratosis.

Consideration of this hypothesis is contained within the thesis and while the recording of malignant change in patients with oral keratoses was undertaken and recognised as important, it was not possible to confirm this hypothesis during the tenure of this study.

CHAPTER 2

CLINICAL METHODS

INVESTIGATION OF PATIENTS

Introduction

This thesis considers clinical and laboratory data accumulated from 159 patients attending Edinburgh Dental Hospital. All patients included in this study were personally examined clinically and investigated, the patients being either self-referred or referred by their General Dental Practioners, General Medical Practioners or Hospital Consultants. A detailed clinical history and examination was recorded for each patient in the hospital records and on a proforma. This proforma was subsequently computerised for the purpose of analysis. Each patient was assigned a study number so as not to contravene the Data Protection Act 1985.

Patients had blood removed for standard haematological and certain biochemical investigations. In addition, venous blood was removed for storage and subsequent analysis as discussed in detail below.

Once the haematological status of the patients was known, deficiencies were investigated and treated appropriately. Thus patients displaying a nutritional or haematological deficiency were further investigated to establish an aetiology for their deficiency. Medical evaluations where appropriate were performed, either by the Department of Haematology in the Royal Infirmary Edinburgh, or by the Department of

Gastroenterology, the Western General Hospital, Edinburgh. Subsequent to these investigations patients with deficiencies were treated for their underlying disease whenever possible before replacement therapy was initiated.

132 patients contributed to the histopathological assessment of the study. Patients presenting with simple frictional or smoking keratoses did not undergo biopsy as this was not thought to be clinically indicated. Additionally one patient (no.126) on anticoagulant therapy was considered unsuitable for biopsy under outpatient conditions.

When necessary, patients were referred to other Departments within the Dental Hospital for specialist dental treatment.

After investigation and diagnosis, the keratoses were treated using accepted methodology including alteration of smoking and drinking habits, surgery and the use of topical and systemic corticosteroids. The controls used for various investigations of the study will be described with the appropriate results section.

a) Clinical History

A clinical history was obtained from each patient; this always included the following :-

A. Clinical disease details including age at onset, duration of illness, involvement of other mucosal surfaces and skin, symptoms, periods of remission and previous treatment provided.

B. Previous dental, medical and drug and habit histories.

Patients currently under care for oral keratoses within the department formed the intial study group. The follow up period for these patients ranged from 3 to 10 years by the end of the 3 year study. Patients joining the study were subsequently followed for the remainder of the study period.

b) Photography

An attempt was made to obtain a photographic record of oral lesions in all patients on their first visit and subsequently when changes were observed. This provided objective records of the clinical appearance as an adjunct to the physical descriptions recorded in the dental notes and on the proforma. In addition photography allowed retrospective analysis of the clinical appearances of each particular lesion and longitudinal assessment of the changes seen during the follow up. 35mm colour film (Kodachrome 25) was used with a Nikon camera and a 100mm lens. A ring flash mounted at the end of the lens provided consistent flash illumination.

c) Physical Examination

The patient's appearance, including facial and limb skin was recorded including their height and weight. Using two dental mirrors and good dental illumination an assessment of the oral mucosa was performed. The mucosa was examined for colour and the extent of mucosal keratinization. For the purposes of tabulating site involvement a

site list was developed (Table 12). A code for the clinical appearance of the keratosis was devised and could thereby be crosstabulated with the site and the diagnostic category.

d) Haematology and Biochemical Evaluation

50mls of peripheral venous blood were taken from each patient and the following parameters were measured by the Haematology Department in the Royal Infirmary, Edinburgh:

1. Haemoglobin, red cell count, haematocrit, mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean cellular haemoglobin (MCH).
2. Total and differential white cell counts.
3. Assessment of a stained blood film.
4. Erythrocyte sedimentation rate (ESR).
5. Serum iron and total iron binding capacity, or serum ferritin.
6. Serum vitamin B12 and serum folate.
7. Blood glucose.
8. Serum complement assessment.

The haemoglobin concentration and the red and white cell counts were measured on a Coulter S counter. The stained blood cell films were examined according to standard methods and differential white cell counts were obtained (374). ESR's were determined using standard techniques (375). Serum iron and total iron binding capacity were measured according to an automated method (376). Iron deficiency was calculated on the basis of iron saturation of transferrin being less

Table 12

Oral Sites of

Keratotic Involvement.

Number	Mucosal Site
1	Vermilion Border
2	Commissures
3	Upper Buccal Sulcus
4	Lower Buccal Sulcus
5	Buccal Surface
6	Buccal Mucosa (3rd Molar Region)
7	Palatal Mucosa
8	Upper Alveolar Ridge
9	Lower Alveolar Ridge
10	Retromolar Region
11	Gingivae
12	Dorsum of Tongue
13	Lateral Border of Tongue
14	Ventral Surface of Tongue
15	Floor of Mouth
16	Pharynx and Fauces

than 16% or a serum ferritin of <10 micrograms per litre for males and <8 micrograms per litre for females on two consecutive occasions (375).

Patients who had values below the normal range were retested to confirm the deficiency before referral for haematological consultation and treatment. Addisonian pernicious anaemia was diagnosed on the basis of blood and bone marrow findings, histamine fast achlorhydria, the presence of gastric parietal cell antibodies, a characteristic Schilling test result and a therapeutic response to Vitamin B12. Patients with persistently reduced serum folate were referred for further investigation which included assessment of dietary intake and exclusion of malabsorption, and jejeunal biopsy.

e) Mucosal Biopsy

Biopsies were obtained from 132 patients for whom there was a clear indication for biopsy to be carried out. Where multiple sites were involved, clinical judgment was used to select a biopsy site that appeared to be representative of the disease generally. In certain cases biopsy of different sites was performed to enable assessment of the histological variation of the presenting clinical features. Previous biopsy material was sometimes available for examination and this helped in the allocation of patients to the appropriate diagnostic category.

Standard clinical procedures were used to obtain the biopsy specimen. Local lignocane anaesthetic solution with 1:80000 Adrenaline was used

to infiltrate the tissues adjacent to the selected site. A 3/0 black silk suture secured the selected tissue which was excised using a number 15 scalpel blade. Closure of the wound was achieved using a 3/0 black silk suture on an atraumatic needle. A chlorhexidine mouth wash 0.2% B.P.C. (Corsodyl) was provided for symptomatic use in the post operative period. Analgesia was obtained post-operatively by using minor analgesics such as aspirin and paracetamol at the patient's discretion. Patients were reviewed after one week for suture removal. Each patient was informed of the results of the histological examination and any necessary treatment was then implemented.

The excised tissue was immediately transported in RMPI 1640 (Flow Laboratories, Rickmansworth, U.K.) with added penicillin and streptomycin at 100ug/ml concentrations. After arrival at the laboratory it was divided into two parts to provide specimens for pathological and experimental purposes. The pathological specimen was submitted for routine histopathological fixation as described in chapter 5. The experimental material was further divided and used for (in vitro) tests of immunocyte function and the remainder stored at -40°C for subsequent immunocytochemistry.

f) Experimental Diagnostic Groupings

After assessment of the histopathological appearances of the biopsy, the microscopic features were collated with the clinical features and the patients were assigned to experimental diagnostic categories. These categories evolved during the three year period when the work for this thesis was being carried out. For the purposes of consistency however it is convenient to describe these categories now before further description and analysis of the work and justification for their adoption is given as the results are displayed and interpreted.

The experimental diagnostic categories were developed from current classifications of oral keratoses adapted to facilitate segregation of the patients according to differences which emerged during this study at clinical, histological and immunological levels.

The 159 patients were divided into two main groups:

1. Infiltrated Keratoses (IK)
2. Non Infiltrated Keratoses (NIK).

A third group was also identified and called Intermediate Infiltrated Keratoses (INT). These will be described in turn.

1) Infiltrated Keratoses (IK)

The criteria for placing patients into the "IK" group was the presence

of a distinct mononuclear cell infiltrate in the lamina propria adjacent to the keratotic epithelium. IK could be further subdivided into Lichen Planus (LP), Infiltrated Leukoplakia (ILK), Discoid Lupus Erythematosus (DLE), Candidal Leukoplakia (CL) and Squamous Cell Carcinoma (SCC). These will be described later.

2) Non Infiltrated Keratoses (NIK)

In the "NIK" group the keratoses were devoid of significant mononuclear cells, the numbers and distribution of such cells being comparable to that seen in normal mucosa. This group could be further subdivided into Non Infiltrated Leukoplakia (NILK), Frictional and Smoking Keratoses (FSK) and Hereditary Keratoses (HK). Again these keratoses will be discussed later.

3) Intermediate Infiltrated Keratosis (INT)

A third group showing intermediate levels of mononuclear cell infiltrate had to be introduced. The infiltrate in these cases appeared distinctly patchy or variable suggesting an intermediate status. Although this may appear a somewhat indefinite group it was considered to be useful to separate these patients from the IK and NIK groups.

Thus patients were assigned to one of the following categories:

1. INFILTRATED KERATOSES (IK)

A) Lichen planus (LP). This category included those patients who had

clinical and histological evidence of lichen planus, that is those patients who had an oral keratosis which clinically was consistent with the features of lichen planus described in the literature review and in addition displayed histological features of lichen planus as described in the microscopic section of Chapter 1. An example of the histopathology for lichen planus is illustrated in Figure 11. The absence of some of the typical histological and clinical features of lichen planus did not exclude a patient from this category but the presence of a band like lymphocytic infiltrate was a prerequisite to inclusion within this category. Due to the subjective nature of the distinction between lichen planus and lichenoid drug reactions, all suspected lichenoid drug eruptions were included in this diagnostic experimental group for purposes of analysis in this thesis.

B) Infiltrated Leukoplakia (ILK). Clinically these lesions were diagnosed as leukoplakia. Histologically the features were variable but a significant mononuclear cell infiltrate was a prerequisite for inclusion in this category (Figure 12).

C) Discoid Lupus Erythematosus (DLE). Clinically these oral lesions often appeared erythematous with a whitish margin. However histological examination showed both superficial and deeper infiltrates in the connective tissue of lymphoid cells, the deeper infiltrates often being perivascular in distribution.

D) Candidal Leukoplakia (CL). Clinically these patients had idiopathic keratoses of a plaque or nodular appearance. Site involvement generally was restricted to the buccal mucosa and

FIGURE 11. Histopathology of Lichen Planus.

(Low Power Magnification)

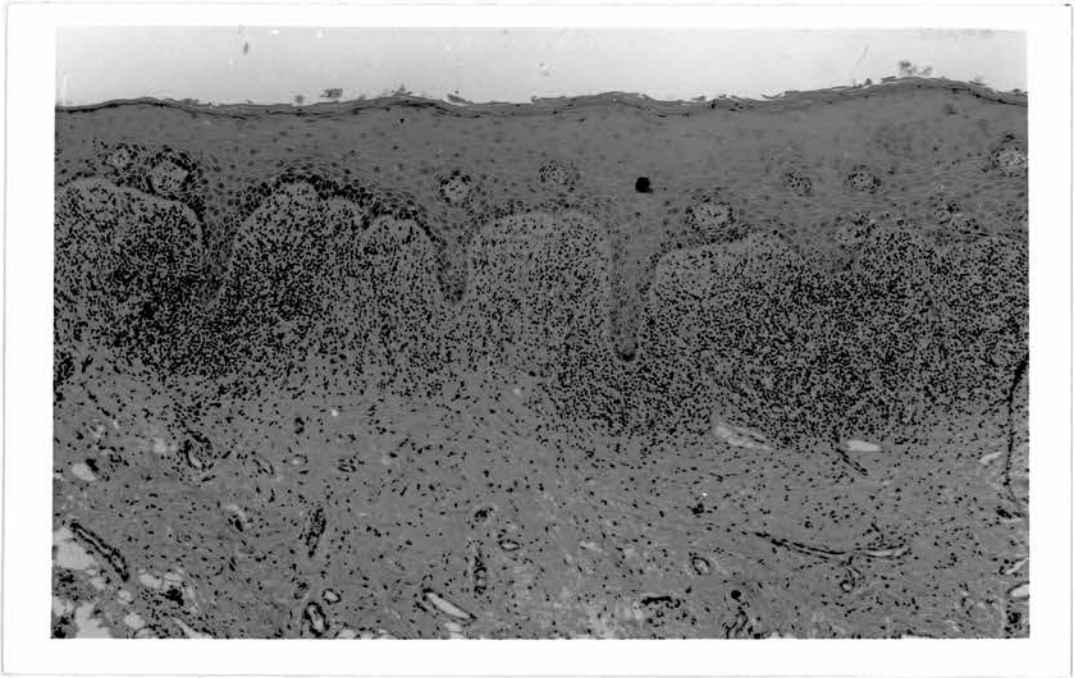
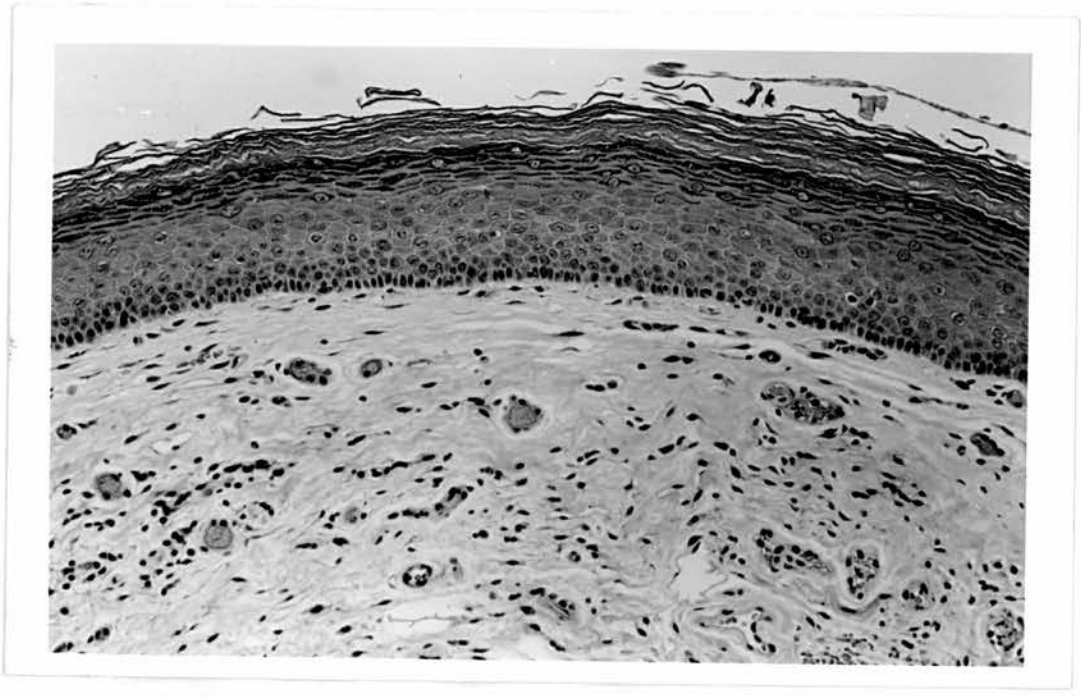


FIGURE 12. Histopathology of Infiltrated Leukoplakia.

(Low Power Magnification)

FIGURE 13. Histopathology of Non Infiltrated Leukoplakia.

(Medium Power Magnification)



commissure. On histopathological examination, using Periodic Acid Schiff (PAS) staining, candidal hyphae and spores could be detected in the superficial layers of the keratin. In addition, dense collections of mononuclear lymphoid cells were present in the connective tissue, with collections of polymorphonuclear leucocytes and micro-abcesses in the epithelium related to the candida hyphae.

E) Squamous Cell Carcinoma (SCC). Cases of squamous cell carcinoma were categorised as infiltrated keratoses in light of the distinct mononuclear cell infiltrates asscciated with the neoplasm.

2. NON INFILTRATED KERATOSES (NIK)

These patients were assigned to one of the following categories:

A) Non Infiltrated Leukoplakia (NILK). These patients presented clinically with idiopathic keratotic lesions (leukoplakia) and histopathological examination of the tissue revealed an oral keratosis with scant or absent mononuclear cell infiltrate (Figure 13).

B) Frictional and Smoking Keratoses (FSK). These keratoses, identifiable clinically, were examined histopathologically in certain cases where doubt existed as to the precise nature of the keratosis. These lesions exhibited a range of clinical signs and displayed only a scant mononuclear cell infiltrate.

C) Hereditary Keratoses (HK). Hereditary keratoses did not display significant infiltrates of lymphoid cells on histological examination

and justified classification as non infiltrated keratoses.

g) Site Involvement

The oral cavity was divided into 16 sites to illustrate the occurrence of keratoses in each particular site. The topographical system used in this thesis was developed from that described by Roed-Peterson and Renstrup (1969) and that used in the WHO oral mucosa manual (377,378). Distinction between left and right was not incorporated into the computation of the oral sites involved due to the complexity involved in analysis of the data. The sites used are defined as follows:

1. Vermilion border. Vermilion border applies to the upper and lower lip area between the labial mucosa and the skin of the lip.

2. Commissure. This is an area of approximately 1.5cms diameter of mucous membrane extending distally from the angles of the mouth.

3. Buccal Sulcus (Upper). The upper buccal sulcus refers to the reflected mucosa from the upper ridge to the flat surface of the buccal mucosa. It includes the labial sulcus as a continuous sheet of mucosa from left to right.

4. Buccal Sulcus (Lower). This, like the upper sulcus, refers to the reflected mucosa between the lower ridge and the flat surface of the buccal mucosa from left to right including the labial sulcus.

5. Buccal Surface. This includes the area between the maxillary and mandibular buccal sulci. It is a rectangular area that extends from the commissure to the anterior faucial pillar.

6. Buccal Surface (Adjacent to Lower Third Molar Region). This describes an area relating to the lower buccal mucosa adjacent to standing or non standing mandibular third molars. This specification was developed to aid topographical details of lichen planus, frequently seen at this site.

7. Palate. This includes the ventral surface of soft and hard palate.

8. Upper Alveolar Ridge. This includes edentulous or dentate ridge from the upper left third molar region to the upper right third molar region.

9. Lower Alveolar Ridge. This likewise includes the ridge from lower left third molar region to the right third molar region.

10. The Retromolar Region. This is a triangular area distal to the lower ridge on the mesial surface of the anterior border of the ascending ramus of the mandible.

11. Gingivae. This included both upper and lower free and attached gingivae adjacent to all standing teeth.

12. Tongue, Dorsal Surface. This includes an area of the tongue from the tip to the circumvalate papillae. This area is bordered by the

lateral surface as noted next.

13. Tongue, Lateral Surface. This is an area covering approximately one centimetre of mucosa that joins the dorsal surface to the ventral surface of the tongue. It comprises the mucosa extending from the dorsal surface to the ventral surface of the tongue.

14. Tongue, Ventral Surface. The ventral surface of the tongue extends from the lateral border of the tongue and includes that mucosa which is raised when the tongue is elevated to the roof of the mouth. The distinction between ventral surface of the tongue and floor of mouth remains controversial.

15. The Floor of the Mouth. This includes the mucosa that comprises the surface of the mucosa in a horseshoe shape that extends from lower left third molar region to the lower right third molar region. This area is bounded by the attached gingival mucosa of the lower ridge and the ventral surface of the tongue.

16. Other Sites. Includes such sites as the pharynx, faucial groove and fauces.

These sites are recorded individually in the results section to describe the distribution of the keratoses examined.

h) Cellobiose/Mannitol Sugar Permeability Test

This test of gastro-intestinal absorption assesses the permeability of the gastro-intestinal system and abnormalities are detected by assessing patient urine for the probe molecules. The tests were all performed under the direction of Dr Anne Ferguson, Professor of Medicine, Western General Hospital. The investigative procedure for a cellobiose/mannitol sugar permeability test is described in detail elsewhere (379). Briefly, following an overnight fast the patient drank the sugar test solution after bladder emptying. The composition of the test solution was as follows; 2 grams mannitol, 5 grams cellobiose, 20 grams lactose, 20 grams sucrose made up to 80mls with boiling water to give an osmolality of approximately 1500 osmols. The sugar test solution was drunk within 5 minutes. The patient fasted for a total of 5 hours after ingestion of the sugar solution with the exception of water, tea or coffee without milk or sugar which were allowed after two and a half hours. All urine passed within 5 hours of the sugar drink was collected, the volume measured and an alliquot stored at -20°C. Mannitol in the urine was measured by the method of Corcorran and Page (1947) (380) and the cellobiose was measured by the method of Strobel et al (1984) (381). For each of the two administered probe molecules, cellobiose and mannitol, the percentage urinary recovery over 5 hours was calculated. The final ratio of percentage recovery of cellobiose to percentage recovery of mannitol was calculated. A cellobiose/ mannitol ratio of $<.037$ was regarded as abnormal (379). The test was carried out on 27 patients with oral keratoses and a comparison group of 31 patients with recurrent aphthous stomatitis.

i) Dermatological Tests

Skin patch tests were carried out on 86 patients who agreed to participate in this part of the study. A panel of Finn chambers (Finn chambers on Scaripon for cutaneous testing. Epitest Ltd Oy., Paijolo 54 SF-04300, Hyryla, Finland) in adhesive tape provided a vehicle for eight commercially available allergens (Table 13). The panel was applied to the patient's arm. In certain cases where a restorative metal might be causing an oral reaction, a powdered form of this metal was also placed in a vaseline base within a Finn chamber and adhered to skin.

Any delayed type hypersensitivity reaction was assessed at 72 hours and patients noticing reaction after this time were requested to identify the number of the test and to contact the department. Reactions occuring within a few hours of the chambers being applied, sufficient for the patient to remove the pannel because of irritation were not considered as hypersensitivity. Photographic verification for the patch test results enabled accurate recording. The tests were later graded on a scale of 0 to 3. The values indicated as follows:

0 - no detectable reaction
1 - mild erythema, restricted to the Finn chamber circumference
2 - raised erythematous reaction within the Finn chamber - palpable
3 - swelling outwith the circumference of the Finn chamber, often with vesicle formation and marked erythema, also extending beyond the circumference of the Finn chamber.

TABLE 13

Substances Used Routinely in Skin Patch Testing

	%	Batch/Lot No.	E No.
1. Nickel Sulphate*	5	42296	E0003
2. Epoxy Resin*	1	42306	E0021
3. Mercury*	0.5	43072	-
4. Potassium Dichromate*	0.5	42288	E0001
5. Cobalt Chloride*	1	42289	E0002
6. Wood Alcohols*	30	41087	E0020
7. Formaldehyde*	1	42519	E0004
8. Acrylic Polymer#	1	-	-

All in white petroleum jelly.

* Trolab, Hermal-Chemie Kurt Herrmann, D-2057, Reinbek b. Hamburg.

Poly-Methylmethacrylate liquid

j) Tobacco and Alcohol Assessment

The clinical history included details of the patients' tobacco and alcohol consumption. The data was collected in an informal manner to encourage patients to state accurately their tobacco and alcohol use, rather than feel it neccessary to underestimate their consumption figures. The patient was asked to recall the number or amount of cigarettes, cigars or tobacco smoked and to recall the amount of alcoholic beverages consumed during a representative week. The patient was further asked to recall the number of years of each habit. It was therefore possible to record the patients who had stopped and those who maintained the respective habits. These details were computerised and a program written to enable extrapolation of the reported daily or weekly consumption of tobacco or alcoholic beverages for the number of years related to the habit. The mean consumption for the various types of tobacco and alcoholic beverages for the respective diagnostic categories could then be expressed as a numerical value. It was also possible to combine the tobacco and alcohol beverage consumption for each patient and thus calculate the mean for the total amount of tobacco and alcohol consumed for the respective categories and similarily for the infiltrated, intermediate and non infiltrated keratoses groups. The assessment of the alcoholic beverage consumption was restricted to the recognised volumes of the different liquids, for example one unit would be equivalent to a half pint of beer or a glass of wine or a quarter gill of spirits. In recording the tobacco consumption, a cigarette or a cigar represented one unit and the amount of tobacco smoked by pipe was recorded in grams per week.

k) Clinical Experimental Requirements

For the lymphocyte transformation and suppressor cell assays, ethical committee approval was obtained to allow age, sex and tobacco habit matched controls to provide venous blood. A consent form was signed and up to 120mls of venous blood was removed from both patient and control using standard techniques which included the use of a butterfly needle.

Simultaneous attendance of the patient and a suitable control could not always be relied upon but the control blood was always obtained during the morning of the same day. Heparin at 20 units per ml of venous blood was added after delivery of venous blood into a 60ml syringe and mixed gently. The blood was then transported to the laboratory immediately for lymphocyte separation.

Fifty controls were also used to provide blood samples for comparison of immunoglobulin levels with the patient group. Ten mls of venous blood was removed from patients attending the casualty service of the Dental Hospital. The blood, once collected, was allowed to clot in standard glass collection tubes and serum subsequently collected and frozen at -40°C.

I) Psychological Assessment

In an attempt to evaluate stress induced anxiety in patients with oral keratoses, the State-Trait Anxiety Inventory (STAI) (382) was used to assess two aspects of anxiety in 126 patients and the data compared with normal values of comparison groups (383). The two aspects of anxiety assessed were state-anxiety, a transitory state characterised by feelings of apprehension and by heightened autonomic nervous system activity, and trait-anxiety, which denotes the personality trait of the individual. Both State anxiety and Trait anxiety were assessed by means of a questionnaire sent out to patients for completion and return. Anonymity was maintained by the use of a coding system. The state-anxiety and trait-anxiety were each measured by 20 self report questions which assess general feelings related to anxiety(A-Trait) and the feelings at the time of completing the questionaire (A-Stait). The range of scoring was from 20 to 80, with a higher score indicating a greater level of anxiety for both state and trait characteristics. The data were compared to reported normal values for medical and surgical patients (382,383).

In addition, the State-Trait Anxiety Inventory (STAI) was used to assess anxiety in 69 patients with recurrent aphthous stomatitis as patients with recurrent oral ulcers are also reported to develop disease associated with stress related anxiety (384).

CHAPTER 3

CLINICAL METHODS 2

TREATMENT AND MANAGEMENT

Preventive Therapy

a) Dental Treatment

Patients attending with identifiable local causes of an oral keratosis had these corrected and patients were subsequently reviewed to evaluate improvement. Patients presenting with an oral keratosis associated with an oral prothesis that was beyond reasonable adjustment, were treated in association with the Prosthodontic Department and again reviewed to evaluate improvements with the new prosthesis. Amalgam restorations adjacent to and associated with oral keratoses were removed and replaced with composite resin restoratives when feasible. In certain cases where cast metal restoratives were related to the keratosis, replacement with cast ceramic crowns was carried out and the keratoses reviewed.

b) Haematinic Therapy

Patients with iron deficiency were treated with ingestable forms of iron over a period of six months after which their iron levels were monitored. Patients with Vitamin B12 and folate deficiency were referred to the Department of Haematology at the Royal Infirmary, Edinburgh where appropriate replacement therapy was implemented and

monitored.

c) Habit Modification

Patient tobacco and alcohol histories were recorded amnestically. All tobacco use was considered to exert an irritant effect on the oral mucosa and patients were encouraged to reduce its use. This, however, proved to be of limited effect although the patients often accepted the reasons for the advice being given.

d) Treatment

Some patients with lichen planus had symptoms of pain, especially with certain foods. Treatment with 0.1% triamcinalone in a dental paste (BPC) was applied to the ulcerated and non ulcerated areas of oral mucosa causing the symptoms. The response was assessed subjectively. If there was no response to triamcinalone, other corticosteroid therapies were used for treatment. Patients were instructed to use an aqueous mouthwash made up using a Betamethasone five gram tablet (BPC) dissolved in a beaker of water, four times daily. The solution was held in the oral cavity for up to five minutes and then expectorated to prevent systemic effects of ingested steroids. In some patients Becotide inhaler spray (BPC) was prescribed and the patient was instructed to direct the spray onto the oral lesion or lesions.

Treatment using systemic corticosteroids in the form of prednisolone (BPC) was initiated in patients with large areas of erosive lichen planus. These (5mg) tablets swallowed twice a day on alternate days

for short periods proved satisfactory in the majority of patients treated in this way. Symptomatic treatment for non erosive and erosive lesions were evaluated and a variety of mouthwashes, including 0.2% chlorhexidine (Corsodyl) and benzydamine hydrochloride 0.15% (Difflam Oral Rinse), were prescribed. In addition, a protective oral gel containing lignocane (Xylodase) was prescribed where painful ulceration restricted eating.

e) Surgical Treatment

Treatment in some patients consisted of excisional biopsy while in others, local surgery was sufficient to completely excise the lesion following incisional biopsy. Patients were referred for treatment to the oral surgery department if a diagnosis of squamous cell carcinoma was confirmed.

f) Follow up

Following diagnosis, all keratoses were followed up and progress was monitored at regular intervals. Where severe ulceration was present and patients were using systemic steroids the reviews were made at two and four weekly intervals. Due to the subjective nature of assessing the response of the disease to treatment quantification of this was not attempted. The response was recorded in the dental notes at review examinations and the patient's perception of response was also often obtained.

g) Data Base

Clinical data on patients in the study were transferred to the Edinburgh University mainframe computer. Care was taken to ensure data protection regulations were adhered to for the interests of these patients in the study. Evaluation of these data primarily involved the use of the Statistical Package for Social Sciences (SPSSX) (385). Advice and assistance in establishing the data base was obtained from the Computer Officer, Barrie Wohlgemuth. Data were put into the computer with the help of the Data Input Service within the University. Control files for data manipulation were developed as the extent of the data base became apparent. Again the author, in association with the computer officer, generated and effected these control files in the analysis of data. The statistical analysis pertinent to the various data will be addressed at the appropriate results section.

CHAPTER 4

LABORATORY METHODS 1 - IMMUNOLOGICAL

a) Nephelometric Quantification of Serum Immunoglobulins

Serum samples, previously described, were obtained from centrifuged clotted whole venous blood from the patient and control groups. The serum was stored at -40°C, until analysis, in sealed 5ml polyethylene tubes.

Samples were then thawed and agitated. Those that appeared excessively turbid due to fat were cleansed using Lipoclean (Fisons Plc, Loughborough, England) in a ratio of 1 to 1.5 parts Lipoclean to serum. The serum was aspirated after centrifugation for 10 minutes at 400g. The serum samples were subsequently retained on crushed ice until dispensed. Total class specific immunoglobulin concentrations for IgG, IgA and IgM were determined using rate nephelometry on a Hyland Laser Nephelometer PDQ. Antiserum specific to human IgG, IgA and IgM was obtained from the Scottish Antibody Production Unit (SAPU) (386) (at Glasgow and West of Scotland Blood Transfusion Service, Law Hospital, Carluke, Lanarkshire, Scotland).

An international reference standard serum (SPS01) was obtained from Milford Ward, Royal Hallamshire Hospital, Sheffield. The concentrations for IgG, IgA and IgM were 9.97, 1.69 and 1.10 grams per litre respectively. Dilutions of SPS01 permitted construction of a standard curve. Controls of pooled sera were also used to evaluate

consistency of method. Reference and test samples were accurately dispensed using automated and programmed diluter dispensers (DILUTREND, Clinicon Manheim GMBH and Microlab-P).

A rack system of 10mm x 75mm Borosilicate glass tubes (Corning N.Y. USA) was arranged to provide a row for each antibody class and a background specimen. To the glass tubes, the reference and test samples were added and subsequently 1ml of the specific antiserum solution.

The antiserum solution consisted of the antiboby in a phosphate buffer saline with 4% polyethylene glycol (PEG) molecular weight 6000 (387). This was made up using 80 grams PEG (Fisons Plc, Loughborough, England), 4.32 grams anhydrous Na_2OH_4, 2.43 grams $Na(H_2PO_4).2H_2O$, 3mls tween 20 and Baxters 0.9% saline to 2 litres. This was Millipore (20um) filtered prior to dispensing appropriate amounts of antisera (Table 16) to separate volumes of the PEG solution. After 30 minutes the PEG was filtered again before dispensing 1ml volumes to the appropriate rows of reference and test specimens. The background row consisted of PEG solution and serum sample, without any antiserum.

The racks of test samples were sealed using Nescofilm (Nippon Shoji Kaisha Ltd, Japan) and agitated. Samples were left for 90 minutes at 15°C to permit immune complex formation. The Hyland nephelometer was set up according to the manufacturer's instructions. The background and test values were computed to provide a value for each specimen for IgG, IgA and IgM. The value in light scatter units is a product of the amount of immune complexes formed which depends directly on the

concentration of antigen (test serum) and excess antibody (SAPU antihuman serum). These values were recorded and the construction of a reference curve permitted test samples to be read in grams per litre.

Statistical analysis of the control sample values, obtained on different days, permitted calculation of the variation of the differences (Table 14). These were within acceptable limits for repeat test procedures (see Table 14).

b) Enzyme-Linked Immunosorbent Assay (ELISA)
Quantification of Class Specific Immunoglobulins

Supernatant samples from (in vitro) immunoglobulin production experiments, described later, were collected and stored at -40°C. The total class specific immunoglobulins, IgG, IgA and IgM, were measured using a one step microtitration plate ELISA technique.

Reagents

A carbonate-bicarbonate buffer (pH 9.6) consisting of 1.59g of Na_2CO_3, 2.93g of $NaHCO_3$ and 0.2g of $NaNO_3$ made up to 1 litre with distilled water, was used to sensitise the plates (387). This was made up, from a concentrated stock, with the class specific sensitising antibody, prior to sensitisation.

TABLE 14

SAPU Anti-Human Immunoglobulin

Class	Source	Dilution	Batch No.
IgG	Sheep (chain specific)	1/100	0368J
IgA	Sheep (chain specific)	1/80	0859J
IgM	Sheep (chain specific)	1/20	5000L

Variation of Control Values

Antibody Class	No. of Tests	Coeff. Var. Diff.*
IgG	8	6.522
IgA	7	10.017
IgM	8	8.450

* Coeff. Var. Diff. - Coefficient of Variation of Differences.

The phosphate buffered saline-tween 20 mixture (PBS-tween) consisted of 8g of NaCl, 0.2g of KH_2PO_4, 2.9g of $Na_2HPO_4.12H_2O$, 0.2g of KCl and 0.5mls of tween-20 in 1 litre of distilled water. The pH of the solution was 7.4.

The alkaline phosphatase conjugate p-nitrophenyl phosphate (Sigma 104 phosphate substrate tablets, 5mg, Sigma Chemical Company) was dissolved in diethanolamine buffer prior to use. A ratio of one 5mg tablet of p-nitrophenyl phosphate to 5mls of diethanolamine buffer at room temperature was used throughout the ELISA tests.

Diethanolamine buffer consisted of 97mls diethanolamine (Fisons Plc, Loughborough, England), 800mls of distilled water, 0.2g of sodium azide and 100mg of $MgCl_2.6H_2O$. A pH of 9.7 was obtained by adding 1M.HCl and the solution was finally made up to 1 litre with distilled water.

Methods

The class specific antibody was attached to the solid phase using a concentration of 30mg/ml protein per 1ml of carbonate buffer. Class specific goat anti-human IgG, IgA and IgM (Sigma Chemical Company) were used to sensitise the appropriate plates.

Disposable polystyrene microtitration plates (Flow Laboratories lot 709211 and Northumbria Biologicals Ltd Product No. M420) consisting of 96 flat-bottomed wells were used as the solid phase. 150 microlitres of prepared buffer were added to all the wells and the plates were

left overnight at 4°C for sensitisation by passive absorption. The plates were subsequently drained, washed three times with PBS-tween and tapped dry. Volumes of 100 microlitres PBS-tween containing 1% newborn calf serum (Flow Laboratories, Rickmansworth, U.K.) were added to all wells. Subsequently the test supernatants and reference samples were added in 50 microlitre volumes. The supernatant samples were each diluted 1:3, 1:9, 1:27, 1:81. The reference sera were used in dilution of 1 in 3000, 1 in 9000 and further serial dilutions. The reference sera, SPS01, were obtained from Milford Ward, Royal Hallamshire Hospital, Sheffield, courtesy of Edinburgh Regional Blood Transfusion Service. The concentrations of IgG, IgA and IgM were 9.97g/l, 1.69g/l and 1.10g/l respectively. The plates were incubated in a humidified atmosphere at room temperature for 90 minutes. The plates were then drained, washed three times in PBS-tween and tapped dry.

100 microlitres of goat anti-human IgG, IgA and IgM alkaline-phosphatase conjugated antisera (Sigma Chemical Company) diluted 1:1000 with PBS-tween containing 1% newborn calf serum were then added to the appropriate plates. These were incubated for 90 minutes before the plates were drained, washed three times and tapped dry.

One hundred microlitres of enzyme substrate p-nitrophenyl phosphate, diluted in diethanolamine buffer was then added to the plate and incubated at room temperature. The colour change, indicated by the substrate degradation, is proportional to the antibody concentration in the test samples. A Titertek Multiscan (Flow Laboratories, Rickmansworth U.K.) was used to read the absorbance of yellow colour

at a wavelength of 405mm. Readings were taken to obtain optimal curves for the dilutions of test and reference samples.

The plate readings were directly transferred and stored on computer 5.25 inch magnetic minidisks (Verbatim Ltd, Limerick, Ireland) using a BBC master computer. The plate readings were subsequently analysed using a microtitre plate computer system (Flow Laboratories Ltd, Rickmansworth and Silicon Lab Ltd, Birmingham) used on a BBC master computer with a second processor. The programme enabled curves for the test samples to be compared to the reference curves for each plate read. The reference curves were run at columns 2 and 12 on the plate and test samples were run in triplicate. The original concentration of the sample could then be calculated.

c) Lymphocyte Transformation

One hundred and twenty millilitres of venous blood was removed from patients and age, sex and tobacco habit matched controls. In the clinic heparin was added at 20 units/ml venous blood (Sodium Heparin, 1000 units/ml, Leo Laboratories Ltd, Risborough, England) and gently mixed. In a unidirectional laminar flow hood (Envair Class II microbiological safety cabinet. BS55726) 25ml volumes of the whole venous blood diluted 1:1 with RMPI 1640 medium (Flow Laboratories, Rickmansworth, U.K.) was layered onto 20ml volumes of ficoll-hypaque lymphocyte separation medium (Flow Laboratories, Rickmansworth U.K.) of specific gravity 1.077 in 50ml disposable polypropylene conical tubes (Flow Laboratories, Rickmansworth, U.K.). The tubes were then centrifuged at 20°C for 30 minutes at 400g. The mononuclear

lymphocytes and monocytes were aspirated from the ficoll-hypaque-plasma interface and washed twice for 10 minutes at 200g in RMPI 1640. The viable leukocytes were counted in a Neubauer counting chamber using 0.2% trypan blue exclusion. They were subsequently adjusted to an appropriate working concentration for the experimental aliquots.

Sterile Microtitre culture plates with 96 flat bottomed wells (Flow Laboratories, Rickmansworth, U.K.) were selected. Cultures of $2x10^3$ leukocytes in a final volume of 0.2ml consisting of 10% foetal calf serum, 100 units/ml of penicillin, 100 units/ml of streptomycin and 2mM.L-Glutamine in RMPI 1640 were dispensed. The cultures were arranged in triplicate.

Mitogens were used to induce leukocyte proliferation at the following concentrations: Phytohaemagglutin (PHA) (Flow Laboratories, Rickmansworth, U.K.) was used at 5ug/ml. Pokeweed Mitogen (PWM) (Flow Laboratories, Rickmansworth, U.K.) was used at 5ug/ml. Concanavalin A (ConA) (Sigma Chemical Company) was used at 10ug/ml (388). Cultures were incubated at 37°C in 100% humidity with 5% CO_2 for 72 hours. Tritiated thymidine, ^{3}H-TdR, (37MBq/ml 1mCi/ml, Amersham International Plc, Buckinghamshire, England) was added at 0.5 uCi per well for the last four hours of incubation.

The cultures were harvested using a semi automatic cell harvester (Titertek Cell harvester, Flow Laboratories, Rickmansworth, U.K.) on to glass fibre filter paper (Titertek, Flow Laboratories, Rickmansworth, U.K.) following a standard sequence of hot, cold and final rinse stages. The filter papers were air dried overnight and

placed in individual 25ml glass vials containing 3mls of scintillation fluid (Optiphase 'X', Fisons Plc, Scientific Equipment Division, Loughborough, England). The Beta radioactivity was subsequently counted in a liquid scintillation counter (Packard tricarb 300) for 60 seconds. The amount of ^{3}H-TdR incorporated in the harvested cells was expressed as net counts per minute (cpm). These values were subsequently stored on the Edinburgh multi access system computer and evaluated using SPSSX (385).

d) Macrophage Mediated Suppression

The macrophage is central to regulatory controls of immune reactions. Lymphocyte proliferation (in vitro) can be suppressed by activated macrophages by their production of oxygen radicals and prostaglandins (389). The role of macrophage mediated suppression was examined in oral keratosis patients and controls to determine whether macrophage stimulation was significant in hyporesponsive proliferation of leukocytes.

Microtiter leukocyte cultures were set up similar to that described for lymphocyte transformation studies. Indomethacin at a final concentration of 50ug/ml (Sigma Chemical Company, USA) and Vitamin E at final concentration of 50ug/ml (Sigma Chemical Company, USA) were added to each culture. The cultures, in triplicate, were harvested after Thymidine labelling and counted as previously described. The data similarly were stored on computer and analysed using the SPSSX package on the University computer.

e) T-Cell Mediated Suppression

The role of T-suppressor cells in lymphocyte function was also investigated. Two aspects were investigated: a) (in vitro) proliferation of T and non-T cells under mitogenic drive of Phytohaemagglutinin, Pokeweed mitogen and Concanavalin-A, b) (in vitro) production of immunoglobulin by plasma cells under the influence of different ratios of T suppressor cells stimulated with the polyclonal activator Pokeweed mitogen.

Methods Leukocytes harvested from the lymphocyte separation procedure were allocated to the separation of T and B cells. T cells were separated from non-T cells by rosetting them with sheep erythrocytes (Tissue Culture Services, Slough, England) treated with 2-amino ethylisothiouronium bromide hydrobromide (AET, Sigma Chemical Company).

In preparation of the treated sheep erythrocytes, the fresh sheep erythrocytes were washed four times in Hanks balanced salt solution HBSS (Flow Laboratories, Rickmansworth, U.K.). The packed cell volume was gently mixed with four volumes of 0.143M AET solution and incubated in a water bath for 15 minutes at 37°C. The cells were then washed four times with ice cold HBSS and finally once with HBSS containing 20% foetal calf serum (FCS) (Flow Laboratories, Rickmansworth, U.K.).

The treated sheep erythrocytes were then added to the allocated mononuclear cells in a ratio of 150:1 to 200:1 and gently mixed. The

erythrocytes were permitted to rosette with the T lymphocytes during a 15 minute incubation at 37°C before centrifugation at 200g for 5 minutes at 4°C. The mixture was then left on ice for 60 to 120 minutes before resuspending the cells and layering on 20mls of ice cold ficoll-hypaque (Flow Laboratories, Rickmansworth, U.K.) in 50ml conical tubes. Centrifugation at 300g for 35 minutes at room temperature provided an upper cell fraction and a pellet of sheep red cell rosettes. The cell rosette fraction was resuspended and sheep erythrocytes lysed by hypotonic shock by placing 9mls of sterile distilled water in the test tube and which was then agitated. 1ml of 10N HBSS (Flow Laboratories, Rickmansworth, U.K.) was immediately delivered into the suspension and agitated again. The cell fractions were twice washed in RMPI 1640 and finally with RMPI 1640 containing 20% FCS to remove cytophilic antibody from the B cell population.

A proportion of the harvest populations of T and non-T cells were allocated to lymphocyte transformation and macrophage suppression experiments. The cells were assessed for viability using trypan blue 0.2% and adjusted to a final concentration of 2×10^3/ml. The remainder of the separated T and non-T cell populations were used to assess T cell suppression of B cell function.

f) T Cell Mediated Suppression of B Cell Function

An indirect method of assessing the immune effects of the immune infiltrate seen in the lamina propria of oral keratoses, is to evaluate the systemic relationship of these cell populations. It was felt that the evaluation of T cell control of B cell function may

provide insight into aetiological aspects of the disease on both a systemic and a local level. Helper and suppressor cells regulate immunoglobulin production, which is a B cell function. Normal immunological events, such as overcoming infections, require a complex interplay between helper and suppressor cells on B cell function.

In an attempt to examine immunoregulatory mechanisms in oral keratoses, the T and non-T cells, separated as described above, were utilised as follows. Cultures were set up in duplicate or triplicate in 6ml round bottomed polyethylene tubes with loose caps (Flow Laboratories, Rickmansworth, U.K.). In RMPI 1640 medium supplemented with penicillin and streptomycin, L-glutamine and 10% newborn calf serum (NCS), the cells were apportioned in T:B ratios of 20:1 to 1:10 to a volume of 1.5mls at a final concentration of $1x10^3$/ml B cells (390). In addition, single cultures of T cells and B cells were performed to confirm adequate subset cell separation.

Mixed mononuclear cell preparations were also alliquoted to assess the immunoglobulin production of the patient, control and lesional cells obtained as described later. Pokeweed mitogen at 50ug/ml was added to the co-cultures and the racks of cultures were incubated for twelve days at 37°C in 100% humidity and 5% CO_2 (400). Infected samples were removed during regular checks on the incubation. The cultures were then centrifuged and supernatants collected and stored at -40°C until analysis of class specific immunoglobulin concentrations using the ELISA method detailed above.

g) Extraction of Lesional Immunocytes

Fresh biopsy tissue removed at the time of venepuncture was used to supply a source of lesional immunocytes. The lymphoproliferative ability of these cells was investigated using the lymphocyte transformation and suppressor cell assay techniques previously described.

The tissue was weighed and then divided to provide histopathological and frozen specimens with the remainder placed in a polystyrene Petri dish (Flow Laboratories, Rickmansworth, U.K.). Using a sterile technique in the lamina flow cabinet, the epithelium and deep fat were removed from the remainder using a scalpel and tweezers. Medium containing 20% NCS was added and the tissue was diced to very fine pieces (less than 1mm cubed). The mixture of fluid and tissue remnants were transferred to a 50ml conical tube and made up to 50mls with rinsings of the Petri dish. This was agitated over a period of 60 minutes and then left vertical to allow larger pieces of tissue to sediment to the bottom of the tube. The supernatant was removed and 25mls layered on 20mls ficoll-hypaque lymphocyte separation medium. Centrifugation at 200g for 30 minutes at room temperature provided a layer of mononuclear cells at the medium, ficoll-hypaque interface. This was carefully aspirated and the cells washed before being assessed for viability using 0.2% trypan blue. The mononuclear cells were identified morphologically and counted using a Neubauer counting chamber.

It was only possible to extract lesional lymphocytes if the biopsy

specimen was sufficiently large, so this investigation was restricted to the larger clinical lesions.

h) Computer and Statistical Methods

The counts per minute of the lymphocyte transformation tests were formatted for computerisation and transferred to the Edinburgh University computer by the Data Preparation Service. This system permitted assessment of data for the subgroups and the total test population. Further data manipulations for net counts per minute (cpm) and stimulation index were evaluated for this study.

CHAPTER 5

LABORATORY METHODS 2 - HISTOPATHOLOGY

a) Initial Preparation of Pathological Tissue

Fresh biopsy specimens were transported from the clinic to the laboratory in RMPI 1640 (Flow Laboratories, Rickmansworth, U.K.) containing 50ug/ml each of Penicillin and Streptomycin at room temperature. On arrival within the hour, the tissue was examined and divided according to experimental requirements. A part of the tissue was always taken for histopathological diagnosis.

For the immunological experiments outlined in the previous section, the tissue was divided to provide two additional pieces. The larger piece was processed as previously described to yield lesional immunocytes. The smaller piece was embedded in O.C.T. compound (Tissue-Tek II, Mile Laboratories, Naperville, Illinois, USA) and frozen on a rapid carbon dioxide delivery chuck. The embedded tissue was stored in 5ml sealed glass vials at -40°C until sectioned on a Slee cryostat (South London Electrical Equipment Co. Ltd.). The dimensions and appearance of the biopsy specimen are dependent on the clinical choice of biopsy site and subsequent surgical procedure.

b) Histopathological Methods

The tissue allocated for diagnostic purposes was processed along with routine specimens in the laboratory. The specimen was fixed in 10% neutral buffered formalin for 24 hours, 5um thick paraffin embedded sections prepared, and stained with haematoxylin and eosin. In addition, Periodic Acid Schiff (PAS) was used routinely.

1) Haematoxylin and Eosin Staining

The following procedure was adopted:

1. Sections were dewaxed then dehydrated in graded alcohols and washed in water
2. Placed in Meyer's haematoxylin for 3 minutes
3. Washed in water
4. Sections were then saturated in lithium carbonate until the haematoxylin turned blue
5. Washed in water
6. Sections then stained in 1% eosin in 1% calcium chloride for 2 minutes
7. Washed in water
8. Dehydrated in graded alcohols and mounted with DPX.

2) Periodic Acid Schiff Staining (PAS)

1. Sections taken to water as in 1. above
2. Treated with 1% periodic acid for 5 minutes

3. Washed in water
4. Stained with Schiff reagent for 10 minutes
5. Washed in water for 10 minutes
6. Counterstain with Meyer's haematoxylin for 30 to 60 seconds and washed with water
7. Blued with lithium carbonate for 10 seconds
8. Washed with water
9. Dehydrated in graded alcohols and mounted in DPX.

c) Immunocytochemical Methods

Serial 5um thick frozen sections were cut on a Slee cryostat (South London Electrical Equipment Co. Ltd.) and collected on poly L-Lysine coated slides (Sigma Chemical Company, USA) (391). The sections were air dried for 60 minutes at room temperature and stored at -40°C in slide trays enclosed in sealed plastic bags to prevent further dessication. The slides were uplifted to a 4°C freezer to enable initial re-equilibration without excess condensation.

The slides were then further dried for 30 minutes before fixation in acetone for 30 minutes (392).

Frozen reactive human lymph nodes were used as tissue controls for the staining technique. These were stored at -40°C before serial 5um cryostat sections were cut and collected on coated slides. The procedure outlined above was also followed for the control sections.

Working titres for the monoclonal antibodies (Table 15) were

determined using a range of monoclonal antibody dilutions from 1:5 to 1:100 on trial mucosal specimens. The monoclonal antibodies were stored in 25 microlitre volumes in sealed 5ml polyethylene tubes (Flow Laboratories, Rickmansworth, U.K.) at -40°C until immediately before use.

The immunoperoxidase technique was carried out as follows. The sections, after dehydration and fixation with acetone, were flooded with rabbit buffer consisting of normal rabbit serum (Scottish Antibody Production Unit, Lanarkshire, Scotland) diluted 1:5 with Tris buffered saline (TBS). The stock TBS formula used was as follows: normal hydrochloric acid (HCl) was added to 500mls of 0.5M tris until pH equalled 7.6. This was then made up to two litres with distilled water and the pH checked. Prior to use the stock was diluted 1:10 with saline. The sections were incubated for 10 minutes in humidified chambers before dabbing off the excess rabbit buffer.

The monoclonal antibodies (Table 15) were made up to their working dilution with rabbit serum buffer and added to the appropriate sections. The slides were incubated for 60 minutes, wiped carefully and washed twice in TBS for 5 minutes.

Rabbit buffer was then pipetted onto the sections and incubated for 10 minutes. This was wiped off and horse radish peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts, Copenhagen, Denmark) was added to the sections in a ratio of 1:20 with rabbit buffer. This was incubated at room temperature in humidified trays for 30 minutes. The slides were then washed twice in TBS for 10 minutes after excess

peroxidase conjugate was removed.

3,3' Diaminobenzidine Tetrahydrochloride (DAB) (Sigma Chemical Company, St Louis, M.O. USA) was added at approximately 1mg/ml to TBS containing 0.01M imidazole (Sigma Chemical Ltd.). Immediately prior to flooding the sections, a drop of 1% hydrogen peroxide (BDH Chemicals Ltd., Poole, England) was added and mixed carefully.

The colour change to brown was observed and, after approximately 5 minutes, the sections were washed in water. Counterstaining of the sections was found unsuitable for the analysis of leukocyte subsets on the Micromagiscan (Joyce Loebl, Gateshead, England), (vide infra) and was not used. The sections were routinely dehydrated in graded alcohols, cleared and mounted for assessment.

For each set of slides, two control slides had the monoclonal antibody omitted to ascertain cross reaction. In addition, for each set of slides, sections of reactive lymph node were stained with monoclonal antibodies, examined for distribution of T and B lymphocytes and to confirm specificity for the appropriate monoclonal antibody (349,393,394). The T4 helper cells were identified predominantly in the paracortical area while B cells were observed in germinal centres.

d) Image Analysis

The micro-magiscan (Joyce Loebl, Gateshead, England) permitted the quantification of positive staining for the sections examined. The procedure involved examination of the sections using a Nikon Optiphot

microscope. This microscope was equipped with a video camera to which adjustments were made for focus and illumination to present a satisfactory image on the computer screen. Following the instructions specified for image analysis, the image was captured and adapted in a systematic fashion for computer evaluation. A low power objective lens (x10) was used to orientate the section. This was followed by using the (x20) objective lens to achieve focus on the area being measured. It was necessary to adjust illumination between each section as the stained area would vary in intensity.

Once the image was seen clearly on the monitor screen, it was captured and the area of detected stain was quantified by the computer. The procedure was repeated for another two fields of that slide for the specific phenotype being examined. The fields were chosen, after initial examination of the section, by selecting three areas in the lamina propria and the epithelium which were representative of the study area as a whole. The total detected area for each of these fields examined were recorded and a mean value obtained for each section. The computer calculation of area was therefore used in each case thereby maintaining identicle parameters for the analysis. This system was repeated for all the stained sections for antibodies T1, T4, T8 and B1. In addition the infiltration into the epithelium by T8 cells was examined by selecting this field on the sections.

TABLE 15

Monoclonal Antibodies

Antibody	Target Cells	Concentration	Class	Laboratory
B1	B cells	1:5	IgG	DAKOPATT
T1	T cells	1:20	IgG	DAKOPATT
T4	Helper T cells	1:5	IgG2b	ORTHO
T8	Suppressor or Cytotoxic T cells	1:30	IgG2a	ORTHO

CHAPTER 6

CLINICAL RESULTS

A total of 159 patients were included in the study of oral keratoses. The patients were assigned an identity number ranging from 1 to 159 which will be used to discuss and examine aspects of the study. Patients were seen prospectively and included in the study group during the three year clinical study which was carried out in the Oral Medicine clinic in Edinburgh Dental Hospital between 1984 and 1987.

EPIDEMIOLOGICAL FEATURES

a) Diagnostic Categories

The patients were allocated to diagnostic categories (table 16) on the basis of clinical and histopathological criteria. The criteria for the diagnostic categories have been stated in Chapter 2, and are reported in two forms; a numerical code used in the computer analysis and a notational abreviation for the diagnosis (Tables 16,18,19).

The division of the patients into the two categories; Infiltrated Keratoses (IK) and Non Infiltrated Keratoses(NIK) is illustrated in Tables 16 and 17. Idiopathic keratosis is further divided into three categories (Table 17) based on the presence or absence of an inflammatory infiltrate associated with the keratosis. The group 12, or Intermediate Infiltrated Keratosis (INT), is included in the IK category as these patients show variable and diffuse infiltrates of

immunocytes in the keratotic lesions during the course of the study. The squamous cell carcinoma category was divided into three groups. The code of 09 refers to patients with squamous cell carcinoma that was seen to arise de novo. The codes 19 and 29 refer to those patients who developed carcinoma in association with lichen planus and leukoplakia respectively (Table 19).

b) Age

The ages of the patients ranged from 15 to 81 years with a mean age of 54 years. The ages of the male patients ranged from 15 to 80 years (mean 52.2 years). The ages of female patients ranged from 17 to 81 years (mean 55.8 years). The ages of the patients in each diagnostic category are displayed in decades (Table 20). The fifth and sixth decades contain the greatest proportion of patients. The mean ages of patients in the diagnostic groups are illustrated in Table 21. All groups have a mean age in the sixth decade except for SCC with a mean age in the seventh decade, and both HK and the CL categories have a mean age in the third decade.

Table 16

Diagnostic Categories for 159 Oral Keratosis Patients

Diagnostic Category	Abreviation	Computer Code
Lichen planus	LP	01
Idiopathic keratosis	IK	02 12 22*
Discoid lupus erythematosus	DLE	06
Candidal leukoplakia	CL	07
Squamous cell carcinoma	SCC	09 19 29*
Frictional, smoking keratosis	FSK	04
Hereditary keratosis	HK	08

* These codes qualify the category of patients within each respective disease. This coding will be explained in Table 19.

Table 17

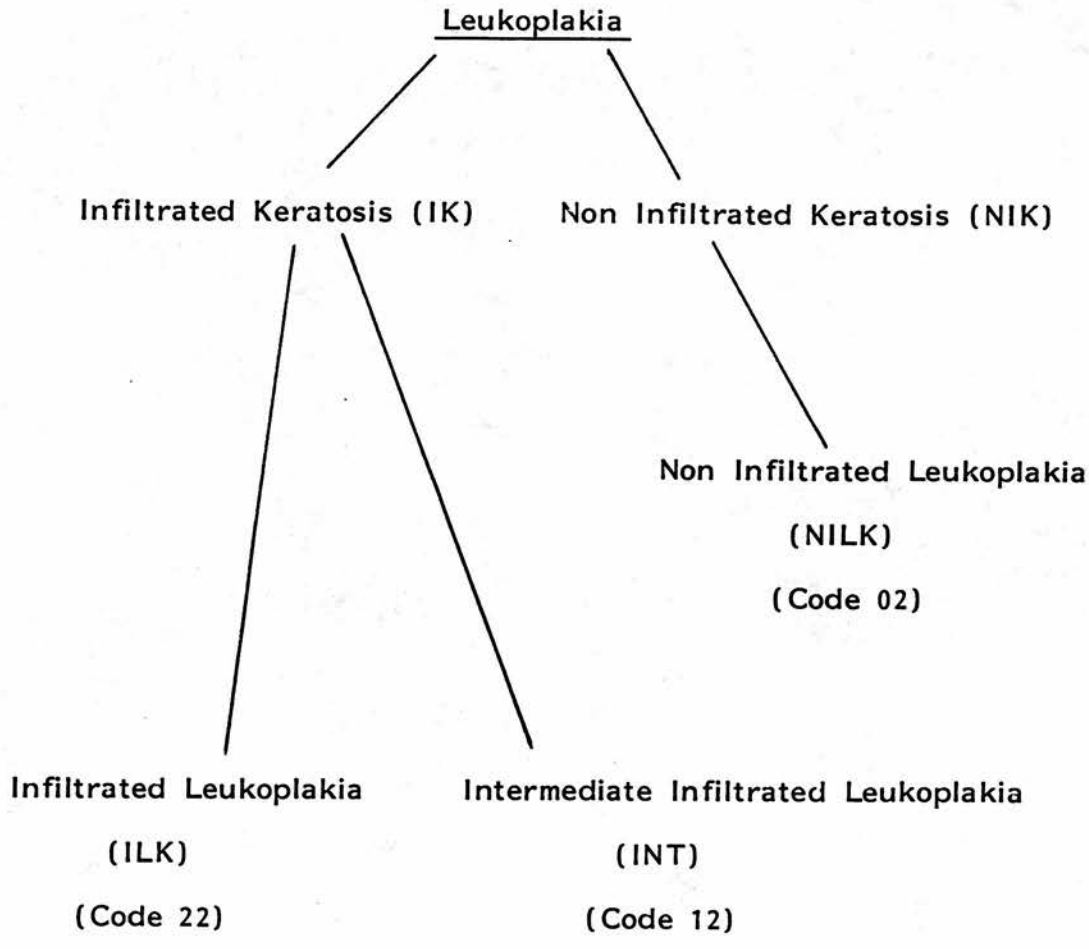

Leukoplakia
Infiltrated Keratosis (IK)
Non Infiltrated Keratosis (NIK)
Non Infiltrated Leukoplakia
(NILK)
(Code 02)
Infiltrated Leukoplakia
(ILK)
(Code 22)
Intermediate Infiltrated Leukoplakia
(INT)
(Code 12)

Table 18

Diagnostic Categories among 159 Patients with Oral Keratoses

Infiltrated Keratoses	Abrev.	Code	No.
Lichen planus	LP	01	59
Int. Infiltrated leukoplakia*	INT	12	9
Infiltrated leukoplakia*	ILK	22	26
Discoid lupus erythematosus	DLE	06	4
Candidal leukoplakia	CL	07	3
Squamous cell carcinoma*	SCC	09	1
Squamous cell carcinoma*	SCC	19	3
Squamous cell carcinoma*	SCC	29	2
Subtotal			107
Non Infiltrated Keratoses			
Frictional, smoking keratosis	FSK	04	24
Non Infiltrated leukoplakia*	NILK	02	26
Hereditary keratosis	HK	08	2
Subtotal			52
Total			159

* See Table 19.

Table 19

Subgroups and Computer Codes for Idiopathic Keratosis and Squamous Cell Carcinoma

Idiopathic Keratosis	Abreviation	Computer Code
Non infiltrated leukoplakia	NILK	02
Int. infiltrated leukoplakia	INT	12
Infiltrated leukoplakia	ILK	22
Squamous Cell Carcinoma	SCC	59
'De Novo'	SCC	09
Assoc. with lichen planus	SCC	19
Assoc. with idiopathic keratosis	SCC	29

Table 20

Age Distribution of 159 Oral Keratosis Patients

AGE*	<20	20+	30+	40+	50+	60+	70+	80+	Total
IK									
LP		5	7	12	15	15	3	2	59
INT					4	4		1	9
ILK		1	2	3	9	7	4		26
DLE				1		2	1		4
CL	1	1		1					3
SCC					2	4			6
Subtotal	1	7	9	17	30	32	8	3	107
NIK									
NILK			2	7	3	8	6		26
FSK	2	1	3	2	5	4	4	3	24
HK		2							2
Subtotal	2	3	5	9	8	12	10	3	54
Total	3	10	14	26	38	44	18	6	159

* Age in decades (1987)

Table 21

Mean Ages for the Diagnostic Categories

IK

Diagnostic Category	Mean Age Yrs.	SD*	No.
LP	51.9	13.7	58
INT	57.8	5.1	8
ILK	56.7	12.6	26
DLE	59.5	13.2	4
CL	29.0	14.9	3
SCC	63.2	5.3	6

NIK

NILK	57.9	13.5	26
FSK	52.5	20.2	22
HK	26.5	2.1	2

* Standard Deviation

c) Height

The mean height for males and females was 173.2cms and 161.5cms respectively (Table 22). The FSK category which had 17 males and seven females, was significantly taller than the LP, ILK, NILK and SCC categories ($p<0.05$).

d) Weight

Patient weights illustrate no significant differences between diagnostic categories (Table 23).

e) Sex

The male to female patient ratio was almost 1:1 with 82 male and 77 female patients. The distribution of male and female patients by age bands is recorded in Table 24. The number of male and female patients in each diagnostic category is displayed in Table 25. There are a higher number of female patients (34) with lichen planus than male patients (25). There are 16 male patients and 10 female patients in the ILK category. The NILK category contains 14 female and 12 male patients. The FSK category however contains a predominance of males (17) compared to females (7).

f) Race

Of the 159 patients with oral keratoses, 153 were North European caucasians, four were Asian and two were African negros.

Table 22

Patient Heights for the Diagnostic Categories

IK

Diagnostic Category	Mean Height (cms)	SD*
LP	167.0	9.3
INT	167.9	13.3
ILK	170.7	8.8
DLE	164.5	5.8
CL	168.0	-
SCC	161.8	11.8

NIK

NILK	165.0	9.9
FSK	173.4	6.5
HK	163.0	-

* Standard Deviation

Table 23

Patient Weights for the Diagnostic Categories

IK

Diagnostic Category	Mean Weight (Kg)	SD*
LP	69.9	12.8
INT	75.0	5.1
ILK	74.1	26.1
DLE	75.3	18.0
CL	73.0	-
SCC	64.8	15.9

NIK

NILK	68.5	16.3
FSK	75.7	14.5
HK	64.0	-

* Standard Deviation

Table 24

Distribution of Male and Female Patients by Age

Age	Male	Female	Total
<20	2*	1	3
20+	6	4	10
30+	7	7	14
40+	18	8	26
50+	19	19	38
60+	21	23	44
70+	6	12	18
80+	3	3	6
Total	82	77	159

* Number of patients.

Table 25

Distribution of Male and Female Patients According to Diagnostic Category

IK

Diagnostic Category	Male	Female	Total
LP	25*	34	59
INT	5	4	9
ILK	16	10	26
DLE	1	3	4
CL	2	1	3
SCC	3	3	6
Subtotal	52	55	107
NIK			
NILK	12	14	26
FSK	17	7	24
HK	1	1	2
Subtotal	30	22	52
Total	82	77	159

* Number of patients.

g) Clinical Lesional Appearance and Site

During the three year period of the study it was recognised that the oral lesions may both arise and disappear. It was therefore useful in recording the topographical involvement of oral keratoses, to attempt to register all sites presenting or arising during the study in a cumulative fashion. The purpose of recording the clinical appearance of the keratoses was to evaluate the range of presenting features for all patients in the study.

1. Oral and Systemic Involvement The keratotic involvement of oral, skin and genital sites was recorded and the data are illustrated in Table 26. Skin involvement was found in 15 of 41 patients with lichen planus. In the INT category, four of the nine patients had dermatological disease. Involvement of the genital mucosa was reported by three patients in the LP category and by one in the CL and NILK categories respectively. The patient with genital mucosa involvement in the CL category had candidal endocrinopathy syndrome and the limited involvement of the nail cuticles was not recorded as dermatological disease.

2. Oral Involvement The distribution of the oral keratoses recorded clinically and photographically was crosstabulated with the diagnostic categories. The Tables 27(i) to 27(xvi) show the range of clinical appearances affecting various oral sites in patients with different diagnoses. The clinical appearances of the lesions were coded and are displayed in Table 27.

The clinical appearance of reticular lichen planus was seen in the diagnostic categories INT, ILK, DLE, NILK and FSK as well as in LP. This feature occurs frequently on the buccal mucosae, sulci and the third molar regions. Furthermore an atrophic appearance of lichen planus was also seen in ILK and DLE for the lower buccal sulcus, buccal mucosa and the third molar region. An appearance of erosive lichen planus occurred frequently on the buccal surface for the LP and ILK categories. Plaque lichen planus, while occurring infrequently for the LP category on buccal sites, was a common presentation on the dorsum of the tongue (10 cases). Furthermore the appearance of plaque lichen planus occurred also in the INT and ILK categories for the buccal surface and lower buccal sulcus. It was also interesting to note plaque lichen planus on the lateral border of the tongue for the LP category. In only one case was bullous lichen planus observed and this occurred in the lower buccal sulcus for the patient in the LP category.

The clinical appearance of leukoplakia in homogeneous forms occurred in the lower buccal sulcus and the buccal surface only for the NILK category. Moreover plaque leukoplakia affected the lower buccal sulcus, buccal surface and third molar regions more frequently for the ILK and INT categories than the NILK category (5:1 cases respectively). Homogeneous leukoplakia occurred infrequently on the floor of mouth and ventral surface of tongue sites for ILK (4 and 2 cases) but frequently for the NILK category (9 and 10 cases). Speckled and nodular leukoplakia occurred infrequently but, when presenting, appeared on the buccal surface for the ILK and INT categories (2 cases). The upper alveolar ridge was a further site

affected with a speckled leukoplakia for ILK (1 case). The dorsum, lateral border and ventral surface of tongue was affected with a plaque leukoplakia in the LP, NILK and INT categories, but not the ILK category. The occurrence of speckled or nodular leukoplakia was not seen on the tongue and floor of mouth (0 cases). Frictional and smoking keratoses were common on the buccal mucosa (12 cases) for the FSK category. The alveolar ridges also were affected with both frictional and smoking keratoses (9 cases).

Squamous cell carcinoma associated with lichen planus as assessed clinically and histopathologically was recorded in four instances in the following regions: lower alveolar ridge, gingivae, lateral border of tongue and the floor of mouth. One patient (no.116) displayed involvement in the first two sites described. Two sites with carcinoma associated with leukoplakia were recorded: buccal surface and the lateral border of the tongue. The occurrence of de novo squamous cell carcinoma was on the lateral border of the tongue. It was interesting to note the clinical presentation of reticular lichen planus on buccal and lingual mucosa within a year of the excision of the neoplasia. The lateral border of the tongue was an oral site common to all three categories of squamous cell carcinoma, endorsing this site as a high risk region of oral mucosa (82,83). Leukoplakia occurs most commonly on the ventral surface of tongue (14 cases) and the floor of mouth (13 cases). Lichen planus most frequently affects the buccal mucosa in the third molar region (43 cases). The other buccal sites also constituted a significant involvement of lichen planus with 38, 34 and 20 cases occurring on the buccal surface, lower and upper buccal sulci respectively.

3. Previous Involvement 44 (29.9%) patients presented with a previous history of oral keratosis of which the involvement ranged from one to thirty one years. In two patients this information was not available. The remaining patients reported no previous involvement prior to the detection and referral of the abnormality by the General Dental or Medical Practitioner.

4. Size of Lesions The area of oral mucosa involved by keratoses was evaluated subjectively. Lesions ranged in size from a few millimetres to areas greater than an estimated 10 square centimetres. Furthermore in one patient (no. 121) disseminated squamous cell carcinoma was present on examination, however the oral lesion was estimated to involve eight square centimetres of oral mucosa including the ulceration. It was appreciated that the size of the keratotic lesions could vary during the course of the study and these estimates of area were taken during the initial examination. The range of lesional area involvement of mucosa is illustrated in Table 28. The greatest proportion of patients had lesional involvement greater than one square centimetre but less than or equal to four square centimetres. A large proportion of patients (58) had oral involvement greater than four square centimetres.

Table 26

Oral, Skin and Genital Involvement for 159 Patients with Oral Keratoses

	Diagnostic Category	Only Oral	Oral+ Skin	Oral+ Genital	Total
IK					
	LP	41*	15	3	59
	INT	5	4		9
	ILK	25	1		26
	DLE	3	1		4
	CL	2		1	3
	SCC	5	1		6
	Subtotal	81	22	4	107
NIK					
	NILK	24	1	1	26
	FSK	24			24
	HK	1	1		2
	Subtotal	49	2	1	52
	Total	130	4	5	159

* Number of patients.

Table 27

Clinical Appearances of Lesions and Respective Codes

	Code	Appearance
Lichen planus	11	Reticular
	12	Atrophic
	13	Erosive
	14	Plaque
	15	Bullous
	19	Squamous cell carcinoma
Leukoplakia	21	Homogeneous
	22	Plaque
	23	Speckled
	24	Nodular
	25	Candidal
	26	Hairy
	29	Squamous cell carcinoma
FSK	41	Smoker's keratosis
	42	Frictional keratosis
DLE	61	Erythematous
	62	Plaque
	63	Ulcerated
Other Diseases	72	Candidal leukoplakia
	81	White sponge naevus
	82	Squamous cell papilloma
	86	Candidosis
	89	Graft
	09	Squamous cell carcinoma (De Novo)

Table 27(i)

Lesional Involvement: Site Vermilion Border

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	3**		2						
21			2				1		
25					1				

* See Table 27.

** Number of patients.

Table 27(ii)

Lesional Involvement: Site Commissure

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	5**		2						
12	1								
13	1								
14	1								
22							2		
25					2		1		
41								1	
42								2	
81									1

* See Table 27.

** Number of patients.

Table 27(iii)

Lesional Involvement: Site Upper Buccal Sulcus

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	20**	2	4	1		1	2		
12	5		1	1					
13	2					1			
21							1		
22			1						
25					1				
41								2	
81									1

* See Table 27.

** Number of patients.

Table 27(iv)

Lesional Involvement: Site Lower Buccal Sulcus

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	34**	5	7	1		1	4	1	
12	5		2	1					
13	2		1			2			
14		1	2						
15	1								
21							3		
22			2						
25					1				
41		1						2	
42								2	
61				1					
81									1
82									1

* See Table 27.

** Number of patients.

Table 27(v)

Lesional Involvement: Site Buccal Surface

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	38**	6	13	1		1	4		
12	9		1	1					
13	6		1			1			
14	1		3					1	
21							1		
22		1	1				1		
24			1						
25					2		1		
26							1		
29							1		
41								4	
42								8	
62				2					
81									1

* See Table 27.

** Number of patients.

Table 27(vi)

Lesional Involvement: Site Buccal Mucosa (3rd Molar Region)

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	43**	4	11	1		1	3		
12	4		2	1					
13	3					2			
14			1				1		
21							2		
22			1						
25					1				
42			2					2	
61				1					
62				1					
81									1

* See Table 27.

** Number of patients

Table 27(vii)

Lesional Involvement: Site Palatal Mucosa

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	3**								
21			1				2	1	
25					1				
41	1	1	1					1	
42							1		
89							1		

* See Table 27.

** Number of patients.

Table 27(viii)

Lesional Involvement: Site Upper Alveolar Ridge

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	3**	1	1						
12	1		1						
21								1	
23			1						
41								2	
42	1							3	

* See Table 27.

** Number of patients.

Table 27(ix)

Lesional Involvement: Site Lower Alveolar Ridge

Clinical Appearance*	Diagnostic Category								
	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	8**		2						
14			1						
19						1			
21		1					2		
22							2		
41								2	
42		1	2				4	2	

* See Table 27.

** Numbers of patients.

Table 27(x)

Lesional Involvement: Site Retromolar Region

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	7**					1			
13						1			
14			1						
21							1		
25					1				
41								1	
42		1	1					2	
62				1					
81									1

* See Table 27.

** Number of patients.

Table 27(xi)

Lesional Involvement: Site Gingivae

Clinical Appearance*	Diagnostic Category								
	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	13**	1	5						
12	3								
13	4								
14							1		
19						1			
21							1		
22							1		
25					1				
41			1				1	1	

* See Table 27.

** Number of patients.

Table 27(xii)

Lesional Involvement: Site Dorsum of Tongue

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	6**	1	4	1		1	2		
12	3								
14	10	1	1						
21			1			1	1		
22	1	1							
25					1				
26							1		
41								2	
61				1					
81									1

* See Table 27.

** Number of patients.

Table 27(xiii)

Lesional Involvement: Site Lateral Border of Tongue

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	22**	4	5	1		1	1		
12	2						1		
13	2		2						
14	3	1							
19						1			
21			2				3		
22	1						1		
25					1				
26							1		
29						1			
42							2	7	
81									1
09						1			

* See Table 27.

** Number of patients.

Table 27(xiv)

Lesional Involvement: Site Ventral Surface of Tongue

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	10**	2	4			2	2		
12	2						1		
13	1					1			
21			4				9		
22		1							
25					1				
26							1		
61				1					

* See Table 27.

** Number of patients.

Table 27(xv)

Lesional Involvement: Site Floor of Mouth

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	2**		1			2			
14			2						
19						1			
21			2				10		
22		1							
61	1								

* See Table 27.

** Number of patients.

Table 27(xvi)

Lesional Involvement: Site Pharynx and Fauces

Clinical	Diagnostic Category								
Appearance*	LP	INT	ILK	DLE	CL	SCC	NILK	FSK	HK
11	3**					1			
25					1				
41								1	

* See Table 27.

** Number of patients.

Table 28

Estimated Areas of Keratotic Involvement of Oral Mucosa

Code	1	2	3	-
No. of Patients	25	67	58	9

Code 1. Lesional involvement equal to or less than one square centimetre.

2. Lesional involvement greater than one square centimetre but equal to or less than four square centimetres.

3. Lesional involvement greater than four square centimetres.

\- No size recording made.

h) Concurrent Oral Disease Concurrent oral disease identified during the clinical examination was recorded (Table 29). It was noted that recurrent oral ulceration was not seen to affect patients attending with oral keratoses. Pigmentation was detected in association with lichen planus in three patients.

i) Concurrent Drug Treatment Medications taken by the 159 patients with oral keratosis were crosstabulated with the diagnostic categories and were cumulated with the following groups of drugs.

1. Non steroidal anti-inflammatory drugs (NSAID)
2. Anti-hypertensive drugs
3. Oral hypoglycaemic drugs
4. Psychotropic drugs
5. Other medications.

It was considered useful to adopt this system of drug categorisation which relates to previous reports and so facilitating comparison of the data (395). Tables 30-32 illustrate the numbers of patients in each diagnostic category taking the specified drug or medication. In Table 32, the lichen planus category was divided in those without erosion (LP) and those with erosion (ELP).

Table 29

Concurrent Disease Detected in 159 Patients with Oral Keratoses

Concurrent Disease	Number
Candidal Infections	
Chronic Candidosis	5
Angular Chelitis	4
Acute Candidosis	1
Median Rhomboid Glossitis	1
Pigmentation	3
Geographic Tongue	1
Xerostomia	1
HIV Infection	1
Erythema Multiforme	1
Total	18

Table 30

Drug History for 159 Patients with Oral Keratoses

	Taking*	NSAID*	A-H*	O-H*	Psych*	Other*	Total Patients
IKP							
LP	33**	6	10	-	6	22	49
INT	8	2	2	1	2	5	9
ILK	15	6	2	-	4	9	26
CL	2	-	-	-	-	-	3
DLE	3	2	1	1	-	1	4
SCC	4	1	-	-	-	3	6
Subtotal	65	17	15	2	12	38	107
NIK							
NILK	18	5	4	1	1	9	26
FSK	12	2	3	-	3	8	24
HK	-	-	-	-	-	-	2
Subtotal	30	7	7	1	4	17	52
TOTAL	95	24	22	3	16	55	159

* Taking- Total Number of Patients on one or more Medications

NSAID - Non Steroid Anti-inflammatory Drugs

A-H - Anti-hypertensive Medication

O-H - Oral Hypoglycaemic Medication

Psych - Psychotropic Medication

Other - Other Medications

** Number of Patients.

Table 31

Drug History for 159 Patients with Oral Keratoses

(Percentage)

	Taking*	NSAID*	A -H*	O-H*	Psych*	Other*
IK						
LP	67	12	20	-	12	45
INT	89	22	22	11	22	56
ILK	58	23	8	-	15	35
CL	66	-	-	-	-	-
DLE	75	50	25	25	-	25
SCC	67	17	-	-	-	50
Subtotal	61	16	14	2	11	36
NIK						
NILK	69	19	15	4	11	35
FSK	50	8	13	-	13	33
HK	-	-	-	-	-	-
Subtotal	58	13	13	2	8	33
TOTAL	60	15	14	2	10	35

* See Table 30

Note : Some patients were taking more than one drug.

Similar percentages for the IK and NIK groups were seen for the six categories of medication. In the IK group, LP had a lower percentage of patients (67%) than the INT (89%), DLE (75%), SCC (67%) and NILK (69%) categories. The LP category also had fewer numbers taking NSAID (12%). This was exceeded by INT (22%), ILK(23%), DLE (50%), SCC (17%) and NILK (19%) categories. 15% of all patients with oral keratoses were taking NSAID. Similarly 14% of these patients were on hypotensive medication. A higher percentage (11%) of IK patients were taking psychotropic drugs than NIK (8%) patients. The INT category had 22% of patients taking psychotropic medication and the ILK (15%) and FSK (13%) categories exceeded the percentage of lichen planus (12%) patients on this form of medication.

The "other" medications category included those that did not qualify for inclusion in the specific categories. Recent history of short term antibiotic therapy was excluded from this, however long term use, in one case was included. This patient (no. 146) was in the NILK category. "Other" medications were taken in high proportions in most categories. The INT category was the highest (56%) with the SCC and LP categories showing 50% and 41% respectively. The ILK and NILK categories had equal percentages of patients using "other" medications (35%).

17 patients in the LP category had erosive lichen planus (ELP). This included three patients who had desquamative gingivitis in addition to lichen planus affecting the buccal mucosa. Table 32 illustrates the numbers and percentages of patients belonging to the ELP and LP categories using medications.

Table 32

Drug Use in Erosive Lichen Planus

	Taking	NSAID	A-H	O-H	Psych	Other	Total Patients
ELP	10*	5	4	0	1	5	17
%	58	29	24	0	6	24	-
LP	23*	1	6	0	5	16	32
%	71	3	19	0	16	50	-

* Number of Patients.

Fewer patients with ELP (58%) were taking medication compared to LP (71%). A greater proportion of those with ELP were taking NSAID (29%) compared to LP (3%) and a higher percentage (50%) in the LP category were taking other medications than the ELP category (24%). Desquamative gingivitis was seen in 17.6% of patients with ELP. This compares closely to values reported by Potts et al 1987 (395).

Patients nos. 81 and 137 were taking insulin for the control of diabetes mellitus. They were from the diagnostic categories INT and LP respectively. Patient no. 81 was taking oral hypoglycaemic medication (chlorpropamide) in addition to the insulin. Furthermore, two patients (nos. 23 and 63) from diagnostic categories DLE and NILK respectively were taking oral hypoglycaemic medication. Apart from these four patients, no further patients were identified as having diabetes mellitus.

j) Erosive Oral Keratoses Erosion or ulceration was not restricted to erosive lichen planus. In addition to the 17 patients presenting with ELP, patients from the ILK, INT, DLE, SCC and NILK categories showed oral erosive lesions (Table 35). 50% of DLE had erosion with LP 35% and INT 22%. There was a similar percentage of patients with erosion in the ILK and NILK categories (15%). The IK group contained 28% of patients with erosions and the NIK group 8%. Within the study population, 21% of patients had erosive lesions.

Table 33

Erosive Lesions

Diagnostic Group	Number	%	Total
LP	17	35	49
INT	2	22	9
ILK	4	15	26
CL	-	-	3
DLE	2	50	4
SCC	5	83	6
Subtotal	30	28	107
NILK	4	15	26
FSK	-	-	24
HK	-	-	2
Subtotal	4	8	52
TOTAL	34	21	159

k) Malignant Change Six patients with oral malignancy contributed to the study. Of these patients, four were seen with neoplasia and two developed neoplasia just prior to the commencement of the study in 1985.

Patient no. 109, female aged 60 years, presented with squamous cell carcinoma of the lateral border of the tongue. This was excised and follow up arranged. Within six months of the excision, histologically proven lichen planus was noted to involve the lateral aspects of the tongue and both left and right buccal mucosae. There was no recurrence during the remaining study period.

Patient no. 121, male aged 70 years, was seen as an inpatient in the Royal Infirmary, Edinburgh. The primary squamous cell carcinoma involved the left lateral border of tongue and the floor of mouth. Metastases were suspected and the tissues were investigated immunologically after radical neck excision. The clinical notes detailed heavy alcohol and tobacco use along with previous oral involvement with leukoplakia. The patient did not survive more than six months after the initial excision.

Patient no. 11, male aged 70 years, had oral leukoplakia involving left and right buccal mucosa. Histopathology initially indicated an infiltrated keratosis (IK) and he was categorised as ILK. Moderate dysplasia was a feature of the initial biopsy. Erosive and hyperkeratotic areas of the buccal mucosa were persistent clinical features but erythroplakia was not considered to be present. Subsequent biopsy of an ulcerated region of the right buccal mucosa

showed severe dysplasia and monthly review continued (Figure 14). A hyperkeratotic lesion involving the right wrist was also biopsied by the dermatopathologist reported as consistent with lichen planus. Outwith the department, systemic prednisolone was prescribed for the patient's oral discomfort. Within six weeks of the second biopsy, an exophytic squamous cell carcinoma of the right buccal mucosa (3cm x 2cm x 1cm) was excised (Figure 15). Node metastases occurred within six months and the patient did not survive a year following removal of the primary carcinoma.

Patient no. 13, female aged 58 years, initially presented with erosive lichen planus involving the floor of mouth, lower alveolar ridge and lateral borders of the tongue before the present study was carried out. A squamous cell carcinoma of the floor of mouth had then developed and subsequent biopsies revealed lichen planus with mild dysplasia. The oral mucosa was seen to improve moderately following the removal of the squamous cell carcinoma. When reviewed as part of the present study the oral lichen planus continued but there had been no recurrence of the carcinoma after five years.

Patient no. 68, female aged 58 years, had atrophic lichen planus involving the left and right buccal mucosae. This tissue became erosive on occasions and allergy to a chrome cobalt denture, as assessed by a patch test, considered to be a further complication. Just prior to the present study squamous cell carcinoma had been removed from the lateral border of the tongue. Following placement of a non allergenic type metal partial denture, the lingual and buccal mucosa was seen to improve markedly. No further complications were

seen during the remainder of the study period.

Patient no. 116, male aged 63 years, presented with an exophytic gingival lesion involving the mandibular left canine and premolar tissues. A similar lesion had previously been removed surgically a year prior to this study, the histopathology of which showed pseudoepitheliomatous hyperplasia with areas of deeper infiltrate of lymphocytes which suggested lichen planus or discoid lupus erythematosus. When the lesion recurred it was surgically removed, together with adjacent teeth. The histological appearance was that of a well differentiated squamous cell carcinoma.

FIGURE 14. Infiltrated Leukoplakia

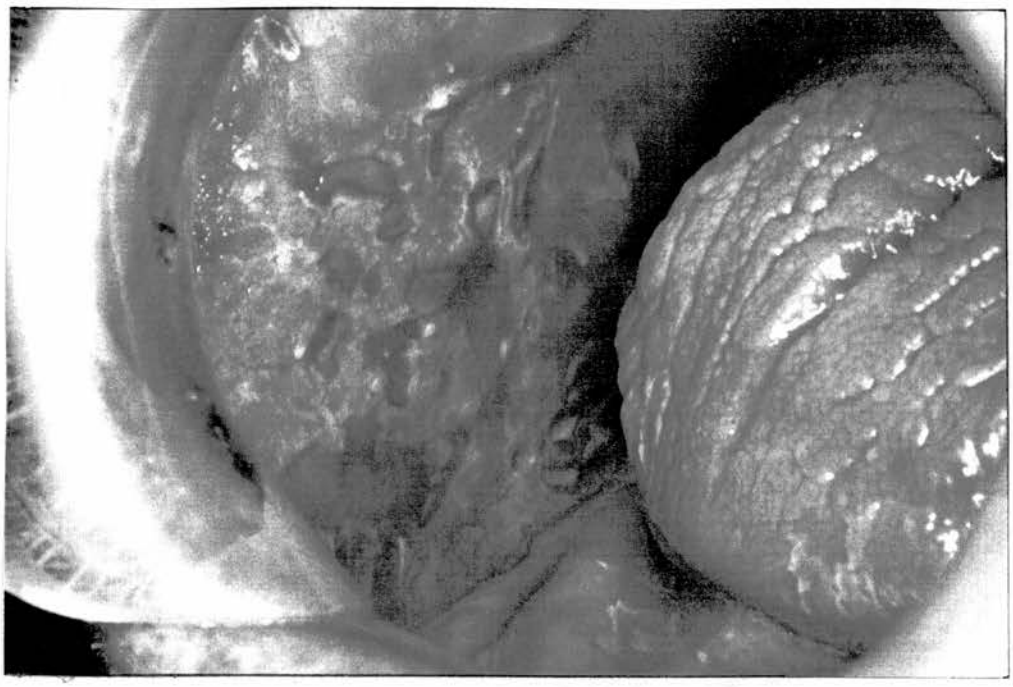
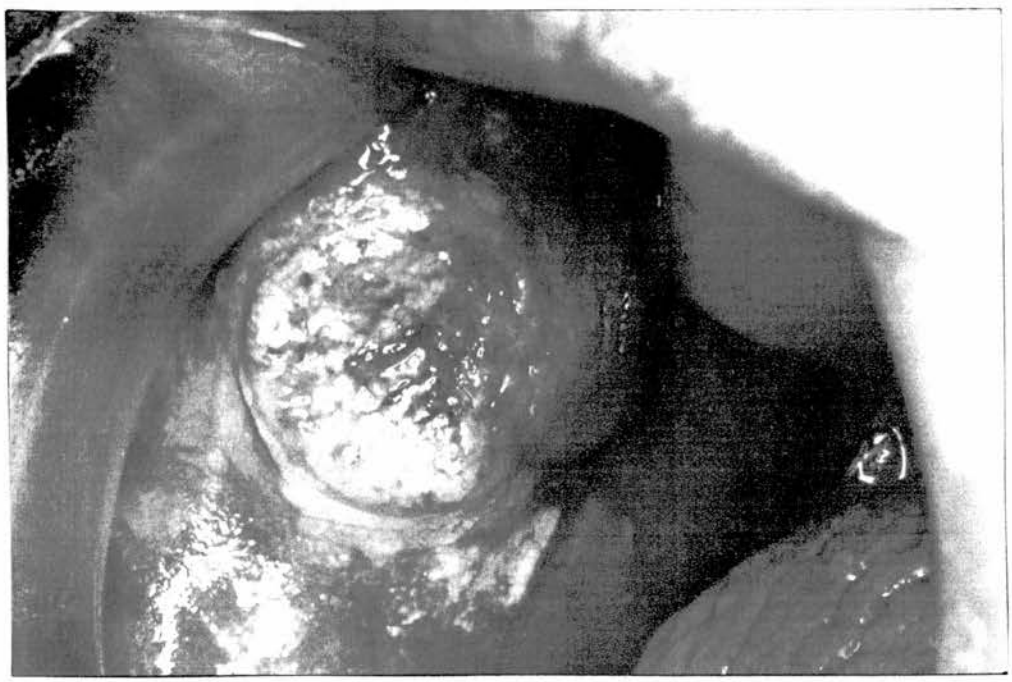


FIGURE 15. Squamous Cell Carcinoma



l) Remission During the study there were spontaneous or therapeutically related remissions of keratoses. This was a subjective assessment of the benefit of clinical management of these patients and cannot be positively related to medication or other treatments used during the study period. Therapeutic measures used in the treatment of these keratoses were altered when initial regimes appeared ineffective in controlling the oral keratosis. Surgical measures were also used to reduce keratoses and routine biopsies played a significant part in the removal of keratotic mucosa. The medications used in the treatment of oral lichen planus are recorded in table 34.

m) Mortality Figures Consistent with the study of this elderly patient population fatalities were recorded. One patient (no.11) with leukoplakia (ILK) and two patients (nos.13,68) with lichen planus died with squamous cell carcinoma. Five patients died due to unrelated causes including gastric carcinoma and hepatic failure. These details are recorded in Table 35.

n) Oral Symptoms These were recorded and ranged from no symptoms to tingling sensations with or without spicy or acidic food or a burning mouth. Seventy eight patients complained of one or more of these symptoms representing 49% of the study population.

Table 34

Steroid Medications used in Treatment of Oral Lichen Planus

Drug	Concentration	Administration
Triamcinalone acetonide	0.1%	Topical
Betamethasone	5 mg	Aqueous mouthwash
Prednisolone	5 mg	Systemic
Triamcinalone acetonide	40 mg/ml	Intralesional

Table 35

Mortality Details

Patient ID	Cause of Death	Age	Diagnostic Category
11	Oral SCC	70	SCC
121	Oral SCC	70	SCC
31	Gastric carcinoma	54	NILK
36	Unknown	68	NILK
57	Unknown	53	LP
61	HIV infection	32	NILK
64	Hepatic failure	67	LP

o) Treatment This was indicated in 106 patients and included the adjustment or renewal of any inadequate dental prosthesis, habit advice, medication or surgical intervention. It was noted that the steroid treatments were varied during the course of the disease thus preventing the tabulation of the response to a particular therapy. Furthermore patients differed in responses to such treatments and in certain cases medication was refused as the patient was prepared to tolerate the oral discomfort.

p) Oral Restorations and Prostheses Oral examination included the inspection of teeth and dental prostheses. In light of reported amalgam allergy and electrogalvanic potentials in certain instances (19,24,27,189-196), a note as to whether amalagam restorations were present was made. Edentulous patients may have had amalgam restorations in the past but this was not investigated and it remains unclear as to whether such a previous history would have any bearing on the disease observed in the present study. The presence of full dentures was recorded and, of these, seven patients had only a full upper set. A further two patients had full upper sets with partial lower dentures. Altogether acrylic dentures were recorded in 45 patients. Two edentulous patients (nos.59,155) did not have dentures or wish dentures.

Amalgam restorations were present in 104 patients and the remaining 10 patients were dentate and amalgam free. These data will be further examined when patch tests to dental materials are reported. Gold restorations in the form of crowns or bridges were noted in 11 patients. Amalgam restorations adjacent and relating to sites of oral

mucosal keratosis were noted in eight patients. While these data are contributory to the clinical details it is unclear whether the restorations have been influential in the oral disease detected.

Furthermore a distinctive keratosis was recorded in patient no. 131 on the left lateral and ventral surface of her tongue in the region adjacent to two dissimilar gold crowns. A galvanic phenomenon was suspected and ceramic porcelain crowns were made to replace the two gold crowns (Figures 16 and 17). Following the replacement of the crowns there was no clinical change in the tongue lesion after one year and this was reduced surgically as it was seen to be spreading anteriorly along the lateral and ventral surface of the tongue. A further case with patient no. 152 suggested a galvanic phenomena where a chrome cobalt partial denture was in contact with an amalgam restored left third molar. The adjacent mucosa improved however, following biopsy in which the affected mucosa was removed under local anaesthetic.

FIGURE 16. Lingual Lichen Planus.

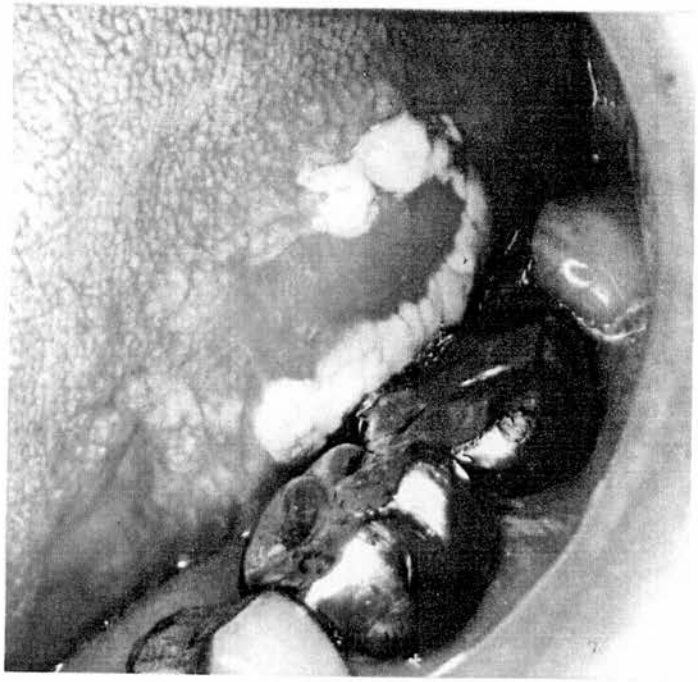
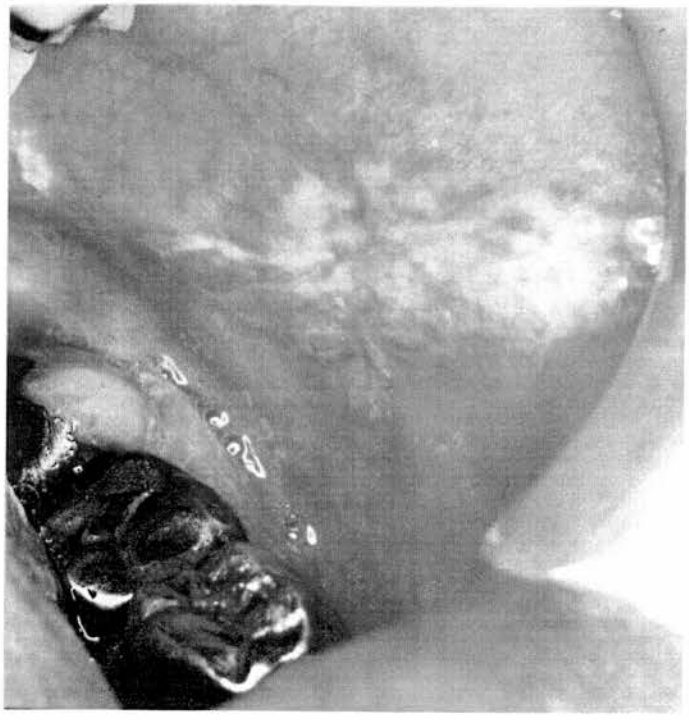


FIGURE 17. Buccal Lichen Planus.



q) Evaluation of Stress Induced Anxiety . Speilberger's State-Trait Anxiety Inventory (STAI) was administered to 126 oral keratosis patients (67 male and 59 female) and compared to data using the same test on recurrent oral aphthous stomatitis patients (52 male and 37 female) at onset of new ulcers (384). Of those patients with oral keratosis, 37 (15 males and 22 females) had lichen planus and 89 (52 males and 37 females) had other oral keratoses. The results are presented in Table 36. There was no significant difference between the scores for patients with lichen planus, with other forms of oral keratoses, with recurrent aphthous stomatitis and with general medical and surgical patients.

Table 36

Trait and State Anxiety Scores

Diagnostic Category	Number	A-Trait#	A-State#
LP	37*	37.9 + 13.4	41.8 + 9.3
OK	89	36.3 + 12.2	41.8 + 11.5
GMS	-	41.9 + 12.7	42.4 + 13.8
RAS	89	38.5 + 10.0	43.6 + 13.4

* Number of Patients

LP - Lichen Planus.

OK - Oral Keratoses (excluding lichen planus).

GMS - General Medical and Surgical Patients (382,383).

RAS - Recurrent Aphthous Stomatitis Patients.

Mean score + standard deviation

r) Cellobiose/Mannitol Sugar Permeability Test Sugar permeability was evaluated in 27 patients with oral keratoses and 31 patients with recurrent aphthous stomatitis (RAS). The RAS patients provided a comparison population for the oral keratoses (OK). The OK included 17 patients with lichen planus, 5 patients with infiltrated leukoplakia (ILK) and 2 with non infiltrated leukoplakia (NILK). There were also 2 patients with frictional or smoking keratoses (FSK) and one patient with squamous cell carcinoma (SCC). These details are summarised in Table 37.

Abnormality was accepted when the cellobiose/mannitol (c/m) ratio was greater than 0.037 and this accounts for the presentation of the data. As illustrated in Table 38, a high proportion of the LP and ILK categories, 65.0% and 60% respectively, have abnormal sugar absorption. Abnormality does not appear to be a feature of the SCC, NILK or FSK categories, with none detected as being abnormal. The RAS group showed 51.9% abnormality. Comparison of the infiltrated and non infiltrated keratoses revealed obvious differences with 64% of patients in the LP and ILK categories being abnormal in sugar absorption and none of the patients in the non-infiltrated keratoses being abnormal. This would suggest that the infiltrated keratoses have abnormal gut permeability and this may be contributory to the oral disease. In almost half of those patients with RAS, abnormality of sugar permeability is also a feature.

Table 37

Patients Participating in Sugar Permeability Tests

	Category	Number
OK	LP	17
	ILK	5
	SCC	1
	NILK	2
	FSK	2
	Total	27
RAS		31
	TOTAL	58

Table 38

Sugar Permeability Ratios for Oral Keratoses and Recurrent Oral Aphthous Stomatitis

	Category	No	Ratio	% Abnormal
IK	LP	11	> 0.037	65.0
		6	< 0.037	
	ILK	3	> 0.037	60.0
		2	< 0.037	
	SCC	1	< 0.037	0.0
NIK	NILK	2	< 0.037	0.0
	FSK	2	< 0.037	0.0
OK (IK+NIK)		14	> 0.037	51.9
		13	< 0.037	
RAS		15	> 0.037	48.3
		16	< 0.037	

OK = Oral keratosis patients

RAS = Recurrent Aphthous Stomatitis Patients

HAEMATOLOGICAL AND BIOCHEMICAL EVALUATION

Mean values for haemoglobin, mean cell volume, white cell count and erythrocyte sedimentation rates are given in Table 39, and total iron binding capacity, serum iron, serum folate, serum Vitamin B12 and a random serum glucose evaluation are given in Table 40.

a) Haemoglobin Four patients (nos.32,33,51,149) had low haemoglobin levels consistent with anaemia. The aetiology of the iron deficiency anaemia was menorrhagia and/or dietary inadequacy. The respective haemoglobin values were 11.9, 6.8, 10.1 and 8.1g/l. Three of the patients were female (nos.32,33,149) and showed classical iron deficiency anaemia with reduced iron levels. Patient no. 32 had only a reduced haemoglobin; patient no. 33 also had a MCV of 57 fl. and a microcytic film and patient no. 149 had a MCV of 71 fl. and also displayed microcytosis. All three women responded to replacement therapy without detectable effect on their oral condition.

The ILK category had a mean haemoglobin of 15.1g/l compared to the lichen planus category of 14.4g/l ($p<0.066$). Patient no. 51, who suffered from candidal endocrinopathy syndrome, also suffered from coeliac disease and pernicous anaemia. Despite parenteral Vitamin B12 supplements his haemoglobin remained at 10.1 g/l.

b) Mean Cell Volume Sixty five (40.9%) patients with oral keratoses showed a raised MCV when compared to normal values for males and females (Table 41). Two patients (nos.10,153) had macrocytosis

associated with deficiencies of folate and Vitamin B12 respectively. Patient no. 66 had a raised MCV of 99 fl. despite iron deficiency. No pathological causes were established for this raised level. Patient nos. 33 and 149 showed reduced MCVs of 57 fl. and 71 fl. respectively. This was consistent with their reduced haemoglobin and anaemia previously reported. The highest MCV recorded was 111 fl. for patient no. 155 in the NILK category. High alcohol consumption was recognised as a contributory factor for this patient.

Significant differences between the diagnostic categories were seen using Student's T-test. The LP category showed a significantly lower MCV value than the ILK, NILK, INT and SCC categories ($p<0.006$). The ILK, NILK and INT categories showed significantly higher values than the FSK category ($p<0.05$). The biological significance of these data remains unclear.

Table 39

Mean Haematological Values in Oral Keratoses

Diagnostic Category	Hb*	MCV**	WBC#	ESR##
IK				
LP	14.4	89.3	6.4	13.0
INT	14.9	92.3	5.7	12.0
ILK	15.1	92.8	6.9	12.0
DLE	13.8	93.5	6.8	27.3
CL	13.0	89.7	7.1	5.0
SCC	14.7	95.3	5.9	22.7
Subtotal	14.5	90.9	6.5	13.7
NIK				
NILK	14.7	94.6	6.7	12.4
FSK	14.5	87.9	6.6	14.1
HK	14.3	90.0	6.2	2.0
Subtotal	14.6	91.4	6.6	12.6
TOTAL	14.6	91.1	6.5	13.4

* Haemoglobin (g/l)

** Mean Cell Volume (fl.)

\# White Blood Cell Count (x1000/ml)

Erythrocyte Sedimentation Rate (mm/hour)

Table 40

Mean Biochemical Values in Oral Keratoses

	TIBC*	Iron	Folate	B12**	Glucose
IK					
LP	63.7	19.5	9.2	503	0.48
INT	59.0	23.8	7.0	382	0.48
ILK	56.2	20.5	7.0	514	0.46
DLE	58.3	19.0	7.4	474	0.56
CL	75.0	38.0	4.7	368	0.46
SCC	63.2	19.8	6.7	499	0.53
Subtotal	61.2	20.4	8.2	492	0.48
NIK					
NILK	60.7	19.5	7.9	380	0.50
FSK	63.5	16.9	8.1	458	0.50
HK	66.5	25.5	7.1	389	0.48
Subtotal	62.1	18.7	8.0	411	0.49
TOTAL	61.5	19.8	8.1	468	0.48

* Total Iron Binding Capacity

** Vitamin B12

Table 41

Mean Cell Volume

	Raised MCV*	Percent
IK		
LP	20**	33.9
INT	4	44.4
ILK	13	50.0
DLE	3	75.0
CL	1	33.3
SCC	3	50.0
Subtotal	44	41.1
NIK		
NILK	15	57.7
FSK	5	20.8
HK	1	50.0
Subtotal	21	40.4
ALL CATEGORIES	65	40.9

* Mean Cell Volume (fl.)

** Number of patients.

c) Erythrocyte Sedimentation Rate The erythrocyte sedimentation rate in the first hour was recorded for patients on initial examination. The mean value for the diagnostic categories ranged from 2mm/hour for the HK category to 27.3mm/hour for the DLE category. These mean values for the diagnostic categories are reported in Table 42.

Male and female patients with values greater than 10 and 15mm/hour respectively were considered to be abnormal and are detailed in Table 42. Female patients within the LP category showed the highest mean ESR of 30.6mm/hour. The lowest mean ESR was 13.8 mm/hour for male patients in the ILK category. The highest recorded ESR of 85mm/hour was recorded for patient no. 64 in the LP category but hepatic failure was present.

Table 42

Abnormal Erythrocyte Sedimentation Rates

		MALE		FEMALE	
		Patient ID	ESR*	Patient ID	ESR*
IK					
	LP	8	35	1	25
		21	16	10	33
		150	19	32	18
				64	85
				80	32
				106	24
				111	16
				118	30
				127	25
				131	29
				140	20
	INT	158	11	81	25
				112	22
	ILK	47	18	24	24
		58	11	125	16
		130	15	129	71
		143	11	138	29
	DLE			23	47
				152	33
	SCC	121	75	68	21
				109	22

Table 42 contd.

Abnormal Erythrocyte Sedimentation Rates

	MALE		FEMALE	
	Patient ID	ESR*	Patient ID	ESR*
NIK				
NILK	35	47	31	17
	146	18	36	27
			63	25
			104	42
FSK	15	13	33	33
	50	66	132	19
	93	16	148	18
	110	11		

* Erythrocyte sedimentation rate mm/hour.

d) Biochemistry 23 patients (14.4%) had iron folate or Vitamin B12 deficiencies, singly or in combination, or were on supplements to correct these deficiencies (Table 43). Eight were male and 15 female. 12 patients had iron deficiency, 2 having iron deficiency anaemia (nos.33,149); 3 were male and 9 were female. Of the 2 with iron deficiency anaemia one (no.33) had a reduced haemoglobin of 6.8g/l and a reduced MCV of 57 and the other (no.149) had a haemoglobin of 8.1g/l and a MCV of 71. Investigation by the Haematology Department, Royal Infirmary, Edinburgh revealed that both were due to dietary deficiency. A third male patient (no.66) with iron deficiency anaemia had a raised MCV but no other abnormality to explain his iron deficiency. The other 2 male patients with iron deficiency (nos.58,59) were alcohol abusers. The remaining woman had latent iron deficiency, either unexplained or associated with menstrual blood loss.

Three patients were folate deficient and one (no.83) was on folate supplements. The 3 untreated patients with folate deficiency were deemed deficient due to dietary causes.

Five patients were Vitamin B12 deficient and 4 (nos.51,64,97,150) were on supplements for previously diagnosed pernicious anaemia. Of the 5 undiagnosed Vitamin B12 deficiencies, 4 were latent and one patient (no.153) had a raised MCV. Two patients (nos.21,153) were subsequently diagnosed as having pernicious anaemia. The remaining 3 were undiagnosed despite further investigation, including absorption studies.

One (no.51) of the 4 treated for Addisonian pernicious anaemia had persistent peripheral blood abnormality with a reduced haemoglobin of 10.1g/l.

Thus only 4 patients (20%) of 19 with previously undiagnosed deficiency showed evidence of peripheral blood changes and the remainder would have been unidentified if investigations had been restricted to routine investigation.

Nonetheless the incidence of deficiencies in this group was not significantly higher than other reported control groups (384) which implies that in this study nutritional deficiencies did not predispose to oral keratoses.

Table 43

Serum Iron, Folate and Vitamin B12 Abnormalities

	ID Number	Iron	Folate	B12
IK				
LP	10		*	
	21	**		**
	32	**		
	53		**	
	64			##
	72			**
	77	**		
	118	**		
	119		**	**
	134	**		
	149	*		
	150			##
ILK	58	**		
	97			##
CL	51			#

See footnote for abreviations

Table 43 contd.

Serum Iron, Folate and Vitamin B12 Abnormalities

	ID Number	Iron	Folate	B12
NIK				
NILK	59	**		
	69			**
	83		#	
	107	**		
	153			*
FSK	33	*		
	66	*		
	151	**		

Footnote

** Deficiency

* Deficiency with anaemia

Supplements

Supplement with anaemia

e) Blood Glucose Blood glucose levels were evaluated for patients as a screening method for diabetes. There was no fasting arranged and the mean values for diagnostic categories are illustrated in Table 40. No abnormally high levels were found and so no patients were found to be diabetic.

f) Serum Complement Evaluation During initial examination peripheral blood was tested for three complement constituents: CH_{50}, C3 and C4. The mean values for these complement components are summarised in Table 44. No significant differences between the diagnostic categories were detected using the Student's t-test. No significant differences were detected between the infiltrated and non infiltrated categories. No individual patient results were outwith the normal range of values.

Table 44

Serum Complement Evaluation

	CH_{50}*	C3**	C4#
IK			
LP	74.2	1.22	0.36
INT	69.8	1.09	0.44
ILK	69.0	1.16	0.37
DLE	89.0	1.31	0.33
CL	84.0	0.92	0.15
SCC	65.3	1.08	0.30
Subtotal	73.0	1.20	0.36
NIK			
NILK	74.6	1.14	0.41
FSK	71.0	1.14	0.39
HK	115.0	1.05	0.37
Subtotal	76.7	1.10	0.40
TOTAL	74.0	1.17	0.37

* CH_{50} The mean total haemolytic capacity of the patients' serum against a human normal pool (%HNP) (normal range 50-150).

** C3 Complement C3 gms/l (Normal range 0.76-1.60).

C4 Complement C4 gms/l (Normal range 0.20-0.65).

g) Autoimmune Evaluation Serum antinuclear factor (ANF) was determined for 74 patients and the results were recorded on a scale of 0 to 3 depending on the reaction observed. Of the patients tested, 38 showed no reaction, 18 were graded at 1, 13 graded at 2 and 5 graded at 3. The diagnostic categories and the ANF determinations are illustrated in Table 45. In the LP category the grades 1, 2 and 3 accounted for 20.6%, 17.6% and 5.9% of patients tested. This compares with the ILK category where the three grades 1, 2 and 3 accounted for 28.6%, 14.3% and 7.1%. The NILK and FSK categories had a higher proportion of patients showing positive reactions. Grades 1, 2 and 3 accounted for 33.3%, 22.2%, 11.1% for NILK and 37.5% and 25.0% for grades 1 and 2 in the FSK category.

Table 45

Autoimmune Evaluation

	0*	1*	2*	3*	Total
IK					
LP	19**	7	6	2	34
INT	2	-	1	-	3
ILK	7	4	2	1	14
DLE	3	-	-	-	3
CL	-	-	-	-	-
SCC	1	1	-	1	3
Subtotal	32	12	9	4	57
NIK					
NILK	3	3	2	1	9
FSK	3	3	2	-	8
HK	-	-	-	-	-
Subtotal	6	6	4	1	17
TOTAL	38	18	13	5	74

* Anti nuclear factor grade.

** Number of patients.

h) Serum Immunoglobulins The main classes of serum immunoglobulins were evaluated and the mean values for the diagnostic categories are presented in Table 46. In general, differences were not obvious from examination of the means obtained for the diagnostic categories. However the lower values seen in the INT category were significantly different from certain other categories when using Student's t-test as follows:

	Class	P Value
INT < LP	IgG	0.008
INT < NILK	IgG	0.021
INT < SCC	IgG	0.002
INT < FSK	IgG	0.000
INT < NILK	IgA	0.021
INT < ILK	IgA	0.052
INT < LP	IgM	0.000
INT < SCC	IgM	0.007

These lower values seen for the INT category for all classes of immunoglobulin indicate humoral differences for this subpopulation of patients with oral keratoses. No significant differences between infiltrated and non infiltrated categories, using Student's t-test, were seen.

Table 46

Serum Immunoglobulins

	Mean Values##		
	G*	A**	M#
IK			
LP	11.7	2.2	1.5
INT	8.8	1.6	0.8
ILK	11.0	2.4	1.4
DLE	10.4	2.0	1.2
CL	11.9	1.5	1.3
SCC	12.6	2.4	1.7
Subtotal	11.5	2.3	1.5
NIK			
NILK	11.8	2.7	1.3
FSK	12.2	2.3	1.2
HK	10.6	3.1	1.6
Subtotal	11.5	2.4	1.2
TOTAL	11.5	2.3	1.4

* Immunoglobulin G

** Immunoglobulin A

\# Immunoglobulin M

Grams per litre

i) Dermatological Tests As illustrated by Tables 47 and 48, dermatological reaction to the panel of standard test materials was uncommon. Notably nickel and mercury showed a greater number of reactions than the other test substances. For LP 13% and 26.3% were positive for nickel and mercury respectively. For ILK the figures were 25% and 37.5% respectively. NILK showed 7.1% and 21.4% positive reaction for nickel and mercury. There was a similar nickel and mercury sensitivity in the FSK category. The typical positive response for mercury is illustrated in Figure 18. Although amalgam restorations were identified adjacent to lichen planus in a number of instances, these results did not support that the mucosal lesions resulted from a hypersensitivity reaction. It was unethical to establish controls for these patch tests but the inclusion of the FSK category enabled comparisons to be made. There were no significant differences between this comparison group (FSK) and other categories for hypersensitivity reaction to the materials tested.

Table 47

Patch Tests to Dental Materials

		Nickel				Mercury				Cobalt			
	0	1	2	3	0	1	2	3	0	1	2	3
IK													
LP	33*	1	3	1	28	10	-	-	37	1	-	-
INT	4	-	-	-	3	1	-	-	4	-	-	-
ILK	12	3	-	1	10	3	2	1	14	2	-	-
DLE	1	-	-	-	1	-	-	-	1	-	-	-
CL	-	-	-	-	-	-	-	-	-	-	-	-
SCC	1	-	-	1	1	-	-	1	2	-	-	-
Sub-Total	51	4	3	3	43	14	2	2	58	3	-	-
NIK													
NILK	13	-	-	1	11	2	-	1	13	1	-	-
FSK	9	2	-	-	9	2	-	-	11	-	-	-
HK	-	-	-	-	-	-	-	-	-	-	-	-
Sub-Total	22	2	-	1	20	4	-	1	24	1	-	-
TOTAL	73	6	3	4	63	18	2	3	82	4	-	-

Code to Reaction after 72 hours

0 - No reaction

1 - Mild erythema and swelling within the disc

2 - Erythema and swelling to margin of disc

3 - Erythema and swelling extending beyond the disc

* Number of patients.

Table 48

Patch Tests to Dental Materials

	Epoxy.		Potass.		Wood Alc.		Form.		Acrylic	
	0	1	0	1	0	1	0	1	0	1
IK										
LP	38	-	38	-	38	-	38	-	38	-
INT	4	-	4	-	4	-	3	1	4	-
ILK	16	-	16	-	15	1	16	-	15	1
DLE	1	-	1	-	1	-	1	-	1	-
CL	-	-	-	-	-	-	-	-	-	-
SCC	2	-	2	-	2	-	2	-	2	-
Subtotal	61	-	61	-	60	1	60	1	60	1
NIK										
NILK	14	-	13	1	14	-	14	-	14	-
FSK	11	-	11	-	11	-	11	-	11	-
HK	-	-	-	-	-	-	-	-	-	-
Subtotal	25	-	24	1	25	-	25	-	25	-
TOTAL	86	-	85	1	85	1	85	1	85	1

Refer to Table 47 for Code description.

Substance Abreviation

Epoxy.	Epoxy resin	Wood Alc.	Wood Alcohols
Potass.	Potassium dichromate	Form.	Formaldehyde
Acrylic	Methylmethacrylate monomer (heat cured type)		

FIGURE 18. Positive Cutaneous Reaction to Mercury.

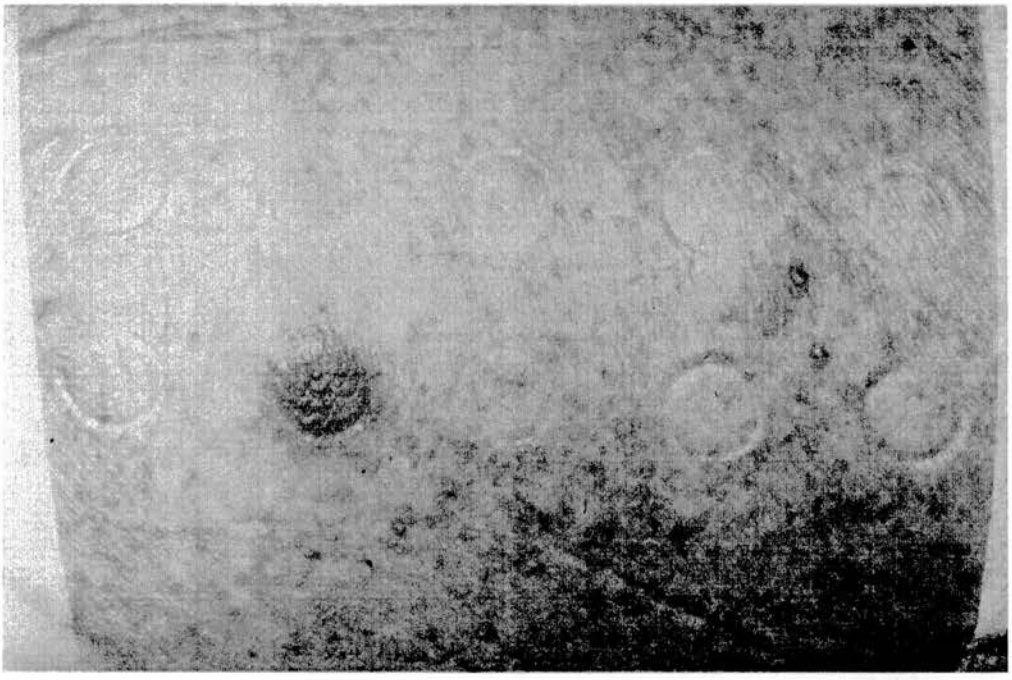
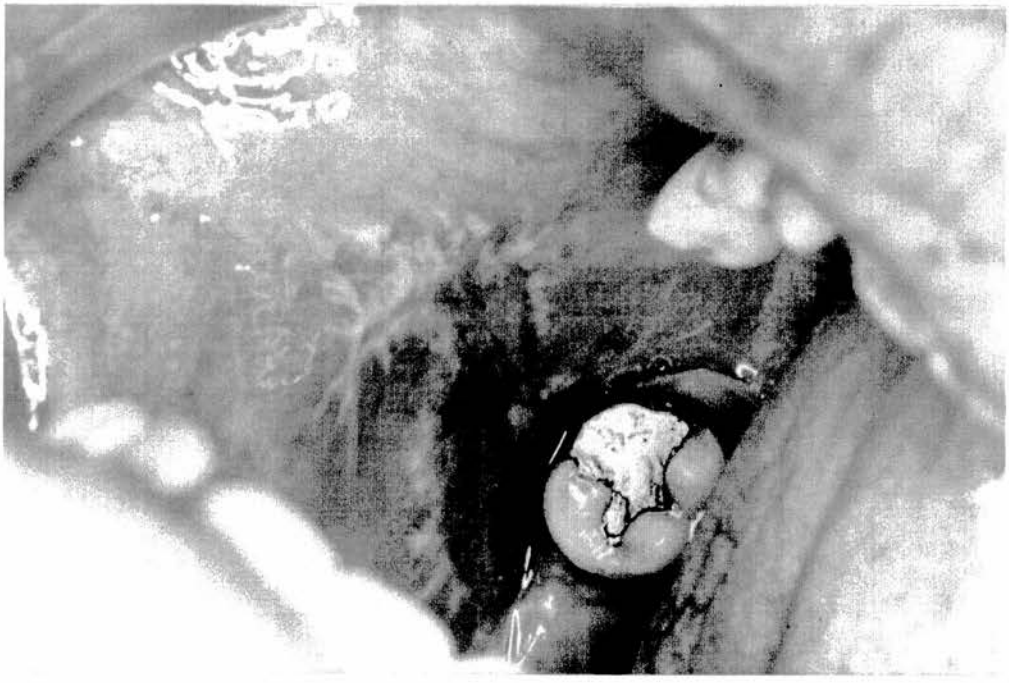


FIGURE 19. Amalgam Adjacent to Oral Lichen Planus.



j) Tobacco Habits

The data detailed in Tables 49 to 55 illustrate the breadth of tobacco habit amongst the study population. Table 49 and 50 shows the tobacco histories of the patients with oral keratoses. 65% of the patients had a positive history of tobacco use and 38% of these patients had stopped the tobacco habit for more than a year during their lives. Non smokers constituted 35% of the patient population. 38% of patients in the immune keratosis (IK) group, compared to 42% in the non immune keratosis (NIK) group, were current smokers.

Table 51 illustrates the distribution of different types of tobacco use amongst the diagnostic categories. Combinations of cigarette, cigar and pipe smoking accounted for a small proportion of the patients.

The distribution of filtered, non filtered and reverse cigarette smoking was also examined and these data are illustrated in Table 51. 73% of patients used tobacco with a filter and 26% of patients had used non filtered tobacco products. This non filter category included those patients who used non filtered cigarettes, cigars or the pipe. Only one patient, in the FSK category, reverse cigarette smoked.

Table 49

History of Tobacco Use

	Never	Smoking	Stopped	+History#	Total
IK					
LP	25*	13	17	30	55
INT	3	5	1	6	9
ILK	5	14	6	20	25
DLE	-	3	1	4	4
CL	1	1	-	1	2
SCC	-	2	3	5	5
Subtotal	34	38	28	66	100
NIK					
NILK	9	12	4	16	25
FSK	9	8	6	14	23
HK	1	1	-	1	2
Subtotal	19	21	9	31	50
TOTAL	53	59	38	97	150

* Number of patients.

Positive History of Tobacco Use

Table 50

Tobacco Use for Filter, Non Filtered and RCS* for Past and Present Smokers

	Never	Filter	Non Filter	RCS*	Total
IK					
LP	25**	23	7	-	30
INT	3	5	1	-	6
ILK	5	14	6	-	20
DLE	-	3	1	-	4
CL	1	1	-	-	1
SCC	-	4	1	-	5
Subtotal	34	50	16	0	66
NIK					
NILK	9	10	6	-	16
FSK	9	10	3	1	14
HK	1	1	-	-	1
Subtotal	19	21	9	1	31
TOTAL	53	71	25	1	97

* Reverse Cigarette Smoking

** Number of patients.

Table 51

Tobacco Use for Different Forms and Combinations of Habit

	Cigs*	Pipe	RCS#	Cigs+ Cigar	Cigar+ Pipe	Cigs+ Pipe	Total
IK							
LP	27**	2	-	1	-	-	30
INT	5	1	-	-	-	-	6
ILK	15	1	-	1	1	2	20
DLE	4	-	-	-	-	-	4
CL	1	-	-	-	-	-	1
SCC	5	-	-	-	-	-	5
Subtotal	57	4	-	2	1	2	66
NIK							
NILK	13	1	-	-	1	1	16
FSK	9	1	1	1	2	-	14
HK	1	-	-	-	-	-	1
Subtotal	23	2	1	1	3	1	31
TOTAL	80	6	1	3	4	3	97

* Cigarettes

\# Reverse Cigarette Smoking

** Number of patients.

For those patients who had stopped smoking tobacco, the numbers and the years of cessation are illustrated in Table 52. There are no obvious differences between the IK and NIK groups for this parameter.

The cigarette, cigar and pipe use for the diagnostic categories is tabulated (Tables 53 to 55). Significant differences between the diagnostic categories using Student's t-test were seen as follows:

Parameter*	Diagnostic Category	P Value
Cigday	LP < ILK	0.007
Gramday	LP < ILK	0.024
Cigyrs	LP < ILK	0.008
Cigall	LP < ILK	0.003
Yrsmoke	LP < SCC	0.002
Cigday	LP < SCC	0.004
Cigyrs	LP < SCC	0.000
Cigyrs	LP < NILK	0.017
Cigall	LP < NILK	0.044
Yrsmoke	NILK < SCC	0.009
Cigyrs	INT < SCC	0.059
Cigyrs	FSK < SCC	0.035

* See glossary of abreviations pXXV

Significant differences appear to exist between the diagnostic categories LP, ILK, SCC and NILK for different aspects of tobacco use. The ILK category smoke cigarettes for significantly more years and this contributed to a significantly higher consumption of cigarette

tobacco than the lichen planus category ($p<0.01$ for these two parameters). In respect of the recognised risk of neoplastic change with prolonged smoking of cigarettes (179,181) the significantly raised consumption of cigarettes both on a daily basis and over a period of years, is significantly higher for the SCC category compared to the LP category ($p<0.001$ for these two parameters). Furthermore, the parameters of a) mean of years smoking cigarettes (cigyrs) and b) the mean total number of cigarettes smoked (cigall), suggests that the smoking patterns of the categories LP, FSK,NILK, ILK and SCC differ in easily recognisable values.

	cigarettes/day	years smoked
LP	10	10
FSK	14	14
NILK	15	17
ILK	20	20
SCC	31	32

This suggests that tobacco smoke plays a part in the aetiology of non infiltrated and infiltrated leukoplakia. A dose relationship appears to be associated for these mean values for these diagnostic categories.

Table 52

Cummulated Years for Cessation of Tobacco Use

	Number	Mean (Yrs)
IK		
LP	17	11.7
INT	1	-
ILK	6	18.8
DLE	1	-
CL	-	-
SCC	3	16.0
Subtotal	28	13.9
NIK		
NILK	4	12.3
FSK	4	13.2
HK	-	-
Subtotal	9	12.7
TOTAL	37	13.6

Table 53

Mean Cigarette Use for Diagnostic Categories

	Number	Cig/Day*	Years*	Cigall*
IK				
LP	54	9.9	9.3	81746
INT	8	11.9	13.8	144859
ILK	24	20.3	19.9	217479
DLE	4	17.3	35.8	195913
CL	2	15.0	17.5	191625
SCC	5	31.0	32.0	389455
mean	(97)	14.1	14.8	144011
NIK				
NILK	25	15.7	17.3	167301
FSK	24	13.9	13.9	124017
HK	2	2.5	5.0	9125
mean	(51)	14.3	15.3	141411
MEAN (Total)	148	14.2	15.0	143132

* Cigall is a mean value for the diagnostic category for total cigarettes consumed. This is calculated by multiplying the number of cigarettes smoked per day by 365 by the years smoked.

Cig/Day x 365 x Years = Cigall.

Table 54

Mean Pipe Tobacco Use for Diagnostic Categories

	Number	gm/Week*	Years*	Gramall*
IK				
LP	54	0.26	0.93	2027
INT	8	6.25	1.75	31937
ILK	24	2.40	3.68	12585
DLE	4	-	-	-
CL	2	-	-	-
SCC	5	-	-	-
mean	(97)	1.27	1.59	6935
NIK				
NILK	25	1.92	1.36	4263
FSK	24	5.73	3.14	28569
HK	2	-	-	-
mean	(51)	3.55	2.10	15002
TOTAL (Total)	148	2.03	1.76	9624

* Gramall is a mean value for the diagnostic category for tobacco used for pipe smoking. This is calculated by multiplying the grams of tobacco used per week by 52 by the years smoked.

Gram/week x 52 x years = Gramall.

Table 55

Mean Cigar Use for Diagnostic Categories

	Number	Cigar/Week*	Years*	Garall*
IK				
LP	54	0.19	-	-
INT	8	-	-	-
ILK	24	2.80	4.24	1164.8
DLE	4	-	-	-
CL	2	-	-	-
SCC	5	-	-	-
mean	(97)	0.82	0.29	297.1
NIK				
NILK	25	0.04	7.99	4.2
FSK	24	-	-	-
HK	2	-	-	-
mean	(51)	0.02	0.86	2.1
MEAN (Total)	148	0.55	0.48	198.8

* Garall is a mean value for the diagnostic category for the total number of cigars smoked. This is calculated by multiplying the number of cigars smoked per week by 52 by the years smoked.

Cigar/week x 52 x years = Garall.

k) Alcohol Habits

Patient alcoholic beverage consumption was recorded and these data are presented in Tables 56 to 61. Of the 143 patients contributing to this information, 62% had used or currently used alcoholic beverages and 38% were non users. The IK group had 51% past or current users of alcohol while the NIK group had 67%. Spirits and beer constituted the highest amounts of alcoholic drinking. Using Student's t-test the following differences between categories are noted:

Parameter*	Diagnostic Category	P Value
Nipyrs	LP <NILK	0.038
Nipyrs	LP <ILK	0.054
Wineyrs	LP <ILK	0.054
Beeryrs	NILK <ILK	0.046
Yrsalk	FSK <ILK	0.056
Yrsalk	FSK <NILK	0.049
Beeryrs	IRK <FSK	0.059
Unwine	SSC <ILK	0.043

* See glossary of abreviations p XXV.

Spirit and beer drinking constitute the more commonly used beverages for the categories of oral keratoses. It is remarkable that both the SCC and the ILK categories have a mean weekly consumption of spirits of 30 and 31 units respectively, comparing with 9 and 12 units for the NILK and LP categories. Furthermore, beer drinking is seen in the ILK,SCC and NILK categories with weekly consumption 13, 15 and 18

units respectively. These results do not achieve statistical significance however a threefold difference between LP and ILK is seen when the mean weekly unit values for spirits and beer are added.

	spirits	+	beer	=	total (mean units/week)
LP	12		3		15
NILK	9		18		27
ILK	31		13		44
SCC	30		13		43

The duration of the use of alcoholic beverages for the categories LP, NILK and ILK was significantly different with LP having the lowest mean and ILK the highest mean ($p<0.05$). These data are similar to the spirit and beer values recorded above, suggesting that comparable patterns of tobacco and alcohol use exist between lichen planus, non infiltrated leukoplakia and infiltrated leukoplakia (see page 232).

Table 56

Patient Alcohol Use for Diagnostic Categories

		Never	User	Stopped	Total
IK					
	LP	25*	27	1	53
	INT	4	4	-	8
	ILK	8	14	3	25
	DLE	4	-	-	4
	CL	1	1	-	2
	SCC	-	3	2	5
	Subtotal	42	49	6	97
NIK					
	NILK	6	17	2	25
	FSK	6	13	-	19
	HK	1	1	-	2
	Subtotal	13	31	2	46
	TOTAL	55	80	8	143

* Number of patients.

Table 57

Cumulated Years for Cesssation of Alcohol Use for the Diagnostic Categories

		Number	Mean (Yrs)
IK			
	LP	2	21.0
	INT	-	-
	ILK	3	4.0
	DLE	-	-
	CL	-	-
	SCC	2	10.5
	Subtotal	7	10.7
NIK			
	NILK	2	12.5
	FSK	-	-
	HK	-	-
	Subtotal	2	12.5
	TOTAL	9	11.1

Table 58

Mean Units of Spirits Consumed for the Diagnostic Categories

		Units/Week*	Years*	Spiritall*
IK				
	LP	11.5	3.3	6151
	INT	2.6	8.2	4797
	ILK	31.4	9.7	14146
	DLE	-	-	-
	CL	2.0	17.5	3640
	SCC	29.6	11.6	16307
	mean	16.2	5.9	8318
NIK				
	NILK	8.5	11.4	9214
	FSK	5.9	8.3	6294
	HK	-	-	-
	mean	7.0	9.8	7607
	TOTAL MEAN	13.3	7.2	8089

* Spiritall is a mean value for the diagnostic category for total units of spirit consumed. This is calculated by multiplying the units consumed per week by 52 by the years of spirit drinking.

Units/week x 52 x years = Spiritall.

Table 59

Mean Units of Beer Consumed for the Diagnostic Categories

		Units/Week*	Years*	Beerall*
IK				
	LP	2.8	2.6	2058
	INT	10.8	4.4	5785
	ILK	12.8	2.2	5809
	DLE	-	-	-
	CL	15.0	17.5	27300
	SCC	15.0	8.0	31200
	mean	6.8	3.1	5270
NIK				
	NILK	17.5	7.4	22526
	FSK	6.5	9.4	9516
	HK	15.0	0.0	-
	mean	12.8	7.9	16173
	TOTAL MEAN	8.7	4.7	8777

* Beerall is a mean value for the diagnostic category for the total number of units of beer consumed. This is calculated by multiplying the units consumed per week by 52 by the years of beer drinking.

Units/week x 52 x years = Beerall.

Table 60

Mean Units of Wine Consumed for the Diagnostic Categories

		Units/Week*	Years*	Wineall*
IK				
	LP	0.8	3.4	602
	INT	-	-	-
	ILK	2.1	10.4	3303
	DLE	-	-	-
	CL	-	-	-
	SCC	0.2	8.0	416
	mean	1.0	5.0	1201
NIK				
	NILK	1.4	3.5	1018
	FSK	0.9	5.0	673
	HK	-	-	-
	mean	1.1	4.0	827
	TOTAL MEAN	1.0	4.7	1083

* Wineall is a mean value for the diagnostic category for the total number of units of wine consumed. This is calculated by multiplying the units consumed per week by 52 by the years of wine drinking.

Units/week x 52 x years = Wineall.

I) Tobacco and Alcohol habits

Table 61 illustrates the mean total amounts of cigarettes, spirits and beer for the categories of keratoses. The "TOTAL" seen in the last column records the mean total value for all the types of alcohol and tobacco consumed by the respective categories. The SCC category had a significantly higher consumption than the NILK category ($p<0.018$) and ILK had a significantly higher consumption than LP ($p<0.004$) for this combination of habits. The highest total value for the SCC category (437×10^3) is almost double the values seen in ILK, CL, NILK, DLE, FSK, and INT (255, 222, 213, 196, 183 and 187×10^3 respectively). The mean value for LP (90×10^3) is less than half of those values seen in the categories detailed above, with lower cigarette consumption the main factor. The mean ages detailed in table 23 suggest that differences in mean age are not significant in the assessment of the tobacco and alcohol habits. As described on page 231-232, the duration of the habit and the number of cigarettes smoked are significant features distinguishing the LP category from the ILK, NILK and SCC categories.

Differences between the Infiltrated Keratoses (IK) and the Non Infiltrated Keratoses (NIK) for the mean values of tobacco, alcohol and the total for these parameters, do not suggest that these habits influence the presence or absence of the infiltrate seen in this keratosis grouping. However the NILK category has a high consumption of beer rather than spirits and the reverse is seen with the ILK and SCC categories, where spirits are consumed in very high ammounts and beer in high amounts (page 238).

Table 61

Mean Cumulation of Tobacco and Alcohol Consumption

		Cigall*	Spiritall*	Beerall*	TOTAL##
IK					
	LP	81.7#	6.2	2.1	90.2
	INT	144.9	4.8	5.8	187.4
	ILK	217.5	14.1	5.8	255.5
	DLE	196.0	-	-	195.9
	CL	191.6	3.6	27.3	222.6
	SCC	389.4	16.3	31.2	437.4
	mean	144.0	8.3	5.3	165.7
NIK					
	NILK	167.3	9.2	22.5	212.8
	FSK	124.0	6.3	9.5	183.1
	HK	9.1	-	-	9.1
	mean	141.4	7.6	16.2	191.2
	TOTAL MEAN	143.3	8.1	8.8	173.9

* Refer to Tables 53,58,59.

\# Values x 10^3.

TOTAL refers to the mean cumulated units of tobacco and alcohol for the diagnostic categories.

CHAPTER 7

HISTOPATHOLOGY RESULTS

1. General Histological Assessment

The male and female distribution, and the biopsy sites for the 132 patients from whom biopsy was carried out, are shown on tables 62 and 63. The assessment of the histopathological features of the 132 biopsies was carried out with particular reference to the following features.

A. Types of keratinization

B. Epithelial thickness

C. Epithelial Dysplasia

D. Epithelial and connective tissue interface

E. Morphological arrangement of inflammatory cell infiltrates

F. Cytological aspects of inflammatory cell infiltrates

G. Blood vessels in the lamina propria

Haematoxylin and eosin stained sections were examined at medium and high power (x10 and x40 objectives) for these features, a minimum of 5 sections at one level being studied. The histological features for this part of the study, were assessed independently without knowing which disease categories the patients had been given. Tables 64-70 are in two forms: a) illustration of the absolute numbers of patients with the specific histological feature for each diagnostic category and b) the percentage, of patients in the diagnostic category, with positive responses for the particular histological feature.

Table 62

Sex Distribution in the Diagnostic Categories for 132 Patients with Oral Keratoses Evaluated Histologically

		Male	Female	Total
IK				
	LP	22*	27	49
	INT	3	3	6
	ILK	16	10	26
	DLE	1	3	4
	CL	1	1	2
	SCC	3	3	6
	Subtotal	46	47	93
NIK				
	NILK	11	13	24
	FSK	10	3	13
	HK	1	1	2
	Subtotal	22	17	39
	TOTAL	68	64	132

* Number of Patients

Table 63

Biopsy Sites for 132 Patients with Oral Keratoses Evaluated Histologically

	Site	IK	NIK	Total	Percentage
1	V. border	-	-	-	-
2	Commissure	3**	1	4	3.0
3	U.B.S.*	2	-	2	1.5
4	L.B.S.*	-	-	-	-
5	Buccal	57	21	78	59.0
6	Buccal 3M	4	-	4	3.0
7	Palate	-	2	2	1.5
8	U.A.R.*	-	-	-	-
9	L.A.R.*	1	1	2	1.5
10	Retromolar	-	-	-	-
11	Gingivae	2	2	4	3.0
12	Tong. Dors.	6	-	6	4.6
13	Tong. Lat.	10	5	15	11.4
14	Tong. Vent.	1	-	1	0.8
15	F.O.M.*	7	6	13	9.9
16	Fauces	1	-	1	0.8
	TOTAL	94	38	132	100.0

* Refer to Chapter 2 for the full description of the 16 sites.

** Number of patients

a) Types of Keratinization

The types of keratinization ie. orthokeratosis, parakeratosis, hyperorthokeratosis, hyperparakeratosis and the presence of a granular layer are given in table 64a and 64b. The predominent feature of keratinization was recorded for each biopsy. Hyperorthokeratosis featured frequently in the INT category, 67%, and to a lesser extent in all other categories. Hyperparakeratosis was seen in 85% of patients in the FSK category and both biopsies (100%) in the HK category. Parakeratosis was a more frequent finding in the IK group, 53%, compared to 13% for the NIK group. However hyperparakeratosis was a more frequent feature in the NIK group (44%) compared to the IK group (24%). The FSK category contributed substantially to the numbers of patients in the NIK group with hyperparakeratosis.

Table 64a

Types of Keratinization in Oral Keratoses

	OK*	PK	HOK	HPK	GRAN	Total
IK						
LP	-	30**	6	13	25	49
INT	-	1	4	1	4	6
ILK	1	11	8	6	15	26
DLE	1	2	1	-	4	4
CL	-	1	-	1	-	2
SCC	-	4	1	1	-	6
Subtotal	2	49	20	22	50	93
NIK						
NILK	5	4	11	4	16	24
FSK	-	1	1	11	2	13
HK	-	-	-	2	-	2
Subtotal	5	5	12	17	18	39
TOTAL	7	54	32	39	68	132

*Code OK - Orthokeratosis

PK - Parakeratosis

HOK - Hyperorthokeratosis

HPK - Hyperparakeratosis

GRAN - Granular layer present

(See text for explanation)

** Number of Patients.

Table 64b

Types of Keratinization in Oral Keratoses

(Percentage)

	OK*	PK	HOK	HPK	GRAN
IK					
LP	-	61	12	27	51
INT	-	17	67	17	67
ILK	4	42	31	23	58
DLE	25	50	25	-	100
CL	-	50	-	50	-
SCC	-	67	17	17	-
Subtotal	2	53	22	24	54
NIK					
NILK	21	17	46	17	67
FSK	-	8	8	85	15
HK	-	-	-	100	-
Subtotal	13	13	31	44	46
TOTAL	5	41	24	30	52

*Code OK - Orthokeratosis

PK - Parakeratosis

HOK - Hyperorthokeratosis

HPK - Hyperparakeratosis

GRAN - Granular layer present

(See text for explanation).

b) Epithelial Thickness

Epithelial thickness was assessed subjectively as normal, hyperplastic, atrophic or with erosion. The range of epithelial thickness is given in tables 65a and 65b. A number of biopsies showed variation in thickness and this is reflected in the disparity between the individual and total numbers.

In the LP category 53% of biopsies were considered to be of normal thickness and 43% to have atrophy. Erosion only affected 10% of the LP category. The INT and SCC categories however showed higher proportions of erosion; 17% and 50% respectively. Erosion or ulceration was an infrequent observation in the NILK category (4%). Atrophy occurred in similar proportions for specimens in the LP, ILK, DLE and SCC categories (approximately 50%). Only in the CL category was atrophy seen in both specimens (100%). As with erosion, atrophy was a less frequent finding in the NIK group (13%) compared to the IK group (45%). Hyperplasia, infrequent in the LP category (14%), was seen in proportionately more cases for ILK (31%), DLE (25%), CL (50%) and SCC (67%). The IK group had 24% of specimens with hyperplasia. In the NIK group, hyperplasia occurred in 54% of cases. Notably the FSK category had a high value of 77% of cases with hyperplasia. This was exceeded only by the HK category where this feature occurred in both cases (100%).

Table 65a

Epithelial Thickness in Oral Keratoses

		Normal	Hyperplasia	Atrophy	Erosion	Number
IK						
	LP	26*	7	21	5	49
	INT	4	1	1	1	6
	ILK	12	8	13	2	26
	DLE	1	1	2	-	4
	CL	-	1	2	-	2
	SCC	-	4	3	3	6
	Subtotal	43	22	42	11	93
NIK						
	NILK	12	9	3	1	24
	FSK	2	10	2	-	13
	HK	-	2	-	-	2
±	Subtotal	14	21	5	1	39
	TOTAL	57	43	47	12	132

* Number of Patients

Table 65b

Epithelial Thickness in Oral Keratoses

(Percentage)

		Normal	Hyperplasia	Atrophy	Erosion
IK					
	LP	53	14	43	10
	INT	67	17	17	17
	ILK	46	31	50	8
	DLE	25	25	50	-
	CL	-	50	100	-
	SCC	-	67	50	50
	Subtotal	46	24	45	12
NIK					
	NILK	50	38	13	4
	FSK	15	77	15	-
	HK	-	100	-	-
±	Subtotal	36	54	13	3
	TOTAL	43	33	36	9

c) Epithelial Dysplasia

Features of epithelial dysplasia have been described in Chapter 1. Epithelial dysplasia is difficult to evaluate objectively so a subjective assessment was made in which five categories were used.

1. No epithelial dysplasia .

2. Minimal epithelial dysplasia minimal features of cellular dysplasia present but not enough to achieve the next category

3. 1/3rd epithelial dysplasia, dysplasia was limited to the basal one third of the epithelium.

4. 2/3rds epithelial dysplasia, dysplasia was limited to the basal two thirds of the epithelium.

5. 3/3rds epithelial dysplasia, dysplasia involved the full thickness of the epithelium.

Difficulties in this evaluation occurred when the epithelium was atrophic and thus the features of epithelial dysplasia would appear to affect a greater proportion of the epithelium. Also where the epithelium contained a thick keratin layer, the part showing dysplasia would thus appear proportionately smaller. The scoring for this parameter of epithelial change was designed to include the four categories of dysplasia, 2 to 5. For Tables 66a and 66b, the data

have been adapted so that the numbers and percentages for each of the four categories of dysplasia indicate the number of patients achieving the stated category of dysplasia.

1. No Epithelial Dysplasia

Epithelial dysplasia could not be identified in the majority of the sections examined.

2. Minimal Epithelial Dysplasia

Minimal epithelial dysplasia was seen in all diagnostic categories except HK (Tables 66a,66b). The highest percentage of biopsies displaying minimal dysplasia (33%) was seen in the INT category. Some specimens in the categories LP, ILK, DLE, NILK and FSK had evidence of a minimal degree of dysplasia.

3. 1/3rd Epithelial Dysplasia

Dysplasia affecting the basal one third of epithelium was seen in a high percentage of ILK and CL sections (39% and 50% respectively). IK showed dysplasia of the basal one third of the epithelium in more cases than NIK (19% and 5%) respectively.

4. 2/3rds Epithelial Dysplasia

Dysplasia involving the basal two thirds of epithelium was seen in the LP, ILK, SCC and NILK categories. While the SCC category contained

the highest proportion (17%), the LP and NILK categories had the lowest with 4% of sections affected. LP, ILK and NILK showed this degree of dysplasia.

5. 3/3rds Epithelial Dysplasia

The presence of dysplasia involving the full thickness of the epithelium was confined to the three patients in the SCC category. The other three patients with sections not showing 3/3rds epithelial dysplasia, had presented with squamous cell carcinoma prior to the study and biopsy during follow-up gave the range of epithelial dysplasia from none to 2/3rds as shown in table 66a.

Table 66a

Epithelial Dysplasia in Oral Keratoses

	None#	Minimal	1/3#	2/3#	3/3#	Number
IK						
LP	33*	9	6	1	-	49
INT	4	2	-	-	-	6
ILK	12	2	10	2	-	26
DLE	3	1	-	-	-	4
CL	1	-	1	-	-	2
SCC	1	-	1	1	3	6
Subtotal	54	14	18	4	3	93
NIK						
NILK	18	3	2	1	-	24
FSK	12	1	-	-	-	13
HK	2	-	-	-	-	2
Subtotal	32	4	2	1	-	39
TOTAL	86	18	20	5	3	132

* Number of Patients

\# Refer to text for explanation

Table 66b

Epithelial Dysplasia in Oral Keratoses

(Percentage)

	None*	Minimal	1/3	2/3	3/3
IRK					
LP	67	18	12	4	-
INT	67	33	-	-	-
ILK	46	8	39	8	-
DLE	75	25	-	-	-
CL	50	-	50	-	-
SCC	17	-	17	17	50
NIRK					
NILK	75	13	8	4	-
FSK	78	8	-	-	-
HK	-	-	-	-	-

* See text for explanation.

d) Epithelial and Connective Tissue Interface

The assessment of the epithelial and connective tissue interface examined three aspects of the histopathology.

1. Liquifactive degeneration
2. Basement membrane thickness
3. Apoptosis

1. Liquifactive Degeneration

Liquifactive degeneration is an appearance produced by oedema in the basal and parabasal layers of the epithelium. Areas of liquifactive degeneration were a feature of all diagnostic categories except HK (Tables 67a and 67b). The LP category contained the highest percentage (71%) showing liquifactive degeneration while other categories such as INT, DLE and SCC had this feature in 67%, 50% and 50% of specimens respectively. The ILK category had 27% showing liquifactive degeneration, representing just over one third of that seen in the LP category. The NILK and FSK categories had lower percentages of liquifaction, 4% and 8% respectively and the HK category had none. There was a large difference in the presence of liquifactive degeneration between the ILK and NILK categories, 27% and 4% respectively, suggesting that an inflammatory infiltrate was related.

2. Basement Membrane Thickness

By basement membrane is meant the interface between the epithelium and

the lamina propria which can be seen as a layer 1-2um thick in normal mucosa with staining such as the PAS reaction. It is a composite structure and includes the basal lamina which can only be seen by electron microscopy. The basement membrane thickness appears greater when an eosinophilic coagulum appears at the junction of epithelium and connective tissue. This is often seen in lichen planus and may also appear in other conditions such as discoid lupus erythematosus. Increased basement membrane thickness was seen in approximately 25% of those patients with lichen planus or discoid lupus erythematosus (Tables 67a and 67b). The ILK category had a lower proportion, 8%, while the INT category failed to demonstrate any thickening of the basement membrane. The NILK category similarly had no thickening of the basement membrane while the FSK category, similar to the ILK category, had 8%. The SCC category demonstrated a thickened basement membrane in one patient (17%).

3. Apoptosis

The presence of hyaline (Civatte) bodies occurring in the lower parts of the epithelium or in the upper parts of the connective tissue is now considered to result from apoptosis. The presence of apoptosis was seen commonly in the LP category (71%), with all other categories in the IK group presenting between 25% and 50% apoptosis (Tables 67a and 67b). In the NILK and FSK categories lower levels of apoptosis were seen, 21% and 23% respectively. Apoptosis was not a feature of the HK category.

Table 67a

Epithelial and Connective Tissue Interface

		Liquifactive Degeneration	TBM#	Apoptosis
IK				
	LP	35*	12	35
	INT	4	-	3
	ILK	7	2	10
	DLE	2	1	1
	CL	-	-	1
	SCC	3	1	3
	Subtotal	51	16	53
NIK				
	NILK	1	-	5
	FSK	1	1	3
	HK	-	-	-
	Subtotal	2	1	8
	TOTAL	57	17	61

* Number of Patients

TBM = Thickened Basement Membrane

Table 67b

Epithelial and Connective Tissue Interface

(Percentage)

		Liquifactive Degeneration	TBM#	Apoptosis
IK				
	LP	71	24	71
	INT	67	-	50
	ILK	27	8	38
	DLE	50	25	25
	CL	-	-	50
	SCC	50	17	50
NIK				
	NILK	4	-	21
	FSK	8	8	23
	HK	-	-	-

TBM = Thickened Basement Membrane

e) Morphological Arrangement of Inflammatory cell Infiltrates

The morphological arrangement of the infiltrate was categorised into four types.

1. A juxta-epithelial infiltrate of inflammatory cells.

2. A band like infiltrate which is a clearly delineated layer from the lower connective tissue region.

3. Inflammatory infiltrates localised to foci of cells irregularly occurring in the lamina propria.

4. The presence of inflammatory infiltrates deep in the connective tissue, often perivascular.

A summary of the results is given in Tables 68a and 68b.

1. Juxta-epithelial Infiltrate

Juxta-epithelial infiltrates occurred in all sections examined from the LP, INT, DLE and SCC categories (100%). The ILK category also had a high proportion of specimens with this feature (62%). Juxta-epithelial infiltrates were also detected in the NILK and FSK categories, 13% and 8% respectively, although these categories by definition would not be expected to have a pronounced inflammatory cell infiltrate.

2. Band Like Infiltrate

92% of samples showed this in the LP category and it was also seen in approximately one third of samples in the INT, ILK and DLE categories. The SCC category had half the samples with this morphological feature while the FSK category had 8%. The subtotals for IK and NIK groups of 63% and 3% illustrate the major difference between these two groups.

3. Localised Foci of Infiltrate

Localised Infiltrates of cells within the lamina propria were uncommon in the LP category (25%) when they related to the localised involvement of the mucosa in reticular and papular lichen planus. Localised infiltrates were seen more frequently in the INT, ILK, DLE, CL and SCC categories (83%, 73%, 75%, 50%, 50% respectively). This contrasted with the localised infiltrates seen in the NILK category where 29% of samples presented this feature. Also one sample in the FSK category demonstrated a localised infiltrate.

4. Deep Infiltrates

The presence of an infiltrate deep in the connective tissue was infrequent in the LP and INT categories (8% and 0%). Other infiltrated keratoses had higher proportions of deep infiltrates identified, notably DLE (75%) where this is one of the diagnostic features of the condition. The ILK, CL and SCC categories had approximately half the samples presenting with deep infiltrates while the NILK category had this feature in 13% of samples. The presence of

deep infiltrates of lymphocytes is a feature occurring more frequently in the IK group than the NIK group, 25% and 8% respectively.

Table 68a

Morphological Arrangement of Inflammatory Cell Infiltrates in Oral Keratoses

	Juxta.#	Bandlike#	Local.#	Deep#	Number
IK					
LP	49*	45	12	4	49
INT	6	2	5	-	6
ILK	16	8	19	12	26
DLE	4	1	3	3	4
CL	1	-	1	1	2
SCC	6	3	3	3	6
Subtotal	82	59	43	23	93
NIK					
NILK	3	-	7	3	24
FSK	1	1	1	-	13
HK	-	-	-	-	2
Subtotal	4	1	8	3	39
TOTAL	86	60	51	26	132

* Number of Patients

Refer to text for explanation of headings

Table 68b

Morphological Arrangement of Inflammatory Cell Infiltrates in Oral Keratoses (Percentage)

	Juxta.#	Bandlike#	Local.#	Deep#
IK				
LP	100*	92	25	8
INT	100	33	83	-
ILK	62	31	73	46
DLE	100	25	75	75
CL	50	-	50	50
SCC	100	50	50	50
Subtotal	88	63	46	25
NIK				
NILK	13	-	29	13
FSK	8	8	8	-
HK	-	-	-	-
Subtotal	10	3	21	8
TOTAL	65	46	39	20

Refer to text for explanation of headings

f) Cytological Aspects of Inflammatory Cell Infiltrates.

The sections were examined for different cellular types that constituted the infiltrates detected. The principle cells detected by histopathological criteria were lymphocytes, plasma cells, macrophages and polymorphonoclear leukocytes. This identification relied on the morphological appearance and staining of the cells using haematoxylin and eosin. Tables 69a and 69b illustrate the range of infiltrates observed in the 132 sections examined.

1. Lymphocytes

Lymphocytes were the most frequently identified cells constituting the infiltrates for the infiltrated and non infiltrated keratoses. In the NILK category one third of the sections revealed lymphocytes (33%) although it must be remembered that by definition NILK lesions contained very few,if any, inflammatory cells.

2. Plasma Cells

No plasma cells were observed in the DLE, FSK and HK categories and were present in only 4% of LP lesions. Plasma cells were present in 83% of the SCC category while the CL and ILK categories had 50% and 23% respectively.

3. Macrophages

Macrophages were seen to be similar to lymphocytes in their

distribution amongst the diagnostic categories. IK and NIK had 96% and 23% of sections with macrophages detected respectively.

4. Polymorphonuclear Leukocytes

Polymorphonuclear leukocytes (PMNL) were detected in many diagnostic categories but always in small percentages. The SCC category had 33% of sections showing PMNL which may be a reflection of the fact that 50% of this category displayed erosion thus permitting superimposed infection.

Lymphocytes and macrophages were the predominant cells in the infiltrates. The presence of PMNL and plasma cells was a much less frequent finding. However the CL, SCC and ILK categories had higher numbers of plasma cells and PMNL cells than other categories. A lower number of plasma cells were found in the INT and LP categories where 17% and 4% of sections showed this feature.

g) Blood Vessels in the Lamina Propria

The final histopathological feature examined was the presence of dilated or increased numbers of blood vessels. It was unclear at the start of the examination what contribution this feature would provide in the overall histopathological evaluation. During preliminary assessments it was thought that the presence of dilated or increased numbers of blood vessels should not be overlooked as increased vascularity was thought to occur. It is difficult to detect an absolute increase in the number of blood vessels as previously

physiologically colapsed vessels may dilate and become visible. A subjective assessment as to whether the vascularity was increased or not was attempted and the results shown in Table 70. The category with the least vascularity was thought to be FSK (8%), contrasting with the other categories which varied between 25% to 50%.

Table 69a

Cell Types in Inflammatory Infiltrates of Oral Keratoses

	Lymph**	Macro#	PMNL##	Plasma cells
IK				
LP	49*	48	2	2
INT	6	6	1	1
ILK	24	23	1	6
DLE	4	4	-	-
CL	2	2	1	1
SCC	6	6	2	5
Subtotal	91	89	7	2
NIK				
NILK	8	7	-	2
FSK	2	2	-	-
HK	-	-	-	-
Subtotal	10	9	-	2
TOTAL	101	98	7	4

* Number of Patients

** Lymph - Lymphocytes

Macro - Macrophages

PMNL - Polymorphonuclear Leukocytes

Table 69b

Cell Types in Inflammatory Infiltrates of Oral Keratoses

(Percentage)

	Lymph**	Macro#	PMNL##	Plasma cells
IK				
LP	100	98	4	4
INT	100	100	17	17
ILK	92	88	4	23
DLE	100	100	-	-
CL	100	100	50	50
SCC	100	100	33	83
Subtotal	98	96	8	2
NIK				
NILK	33	29	-	8
FSK	15	15	-	-
HK	-	-	-	-
Subtotal	26	23	-	5
TOTAL	77	74	5	3

** Lymph - Lymphocytes

Macro - Macrophages

PMNL - Polymorphonuclear leukocytes

Table 70

Sections Detected with Increased vascularity in the Connective Tissue

	Number	Percentage
IK		
LP	22	45
INT	2	33
ILK	13	50
DLE	2	50
CL	-	-
SCC	3	50
Subtotal	42	45
NIK		
NILK	6	25
FSK	1	8
HK	-	-
Subtotal	7	18
TOTAL	49	35

CHAPTER 8

IMMUNOCYTOCHEMISTRY RESULTS

Immunocytochemistry was used for investigation of the subsets of immunocytes present in the biopsies when the biopsies were of sufficient size to allow both histolopathology and immunocytochemical analysis. 28 patients provided biopsies for this investigation and 2 of these patients provided biopsies on two separate occasions, providing 30 sets of stained sections (Table 71). The purpose of this study was to compare the quantity of different immunocytes present in both the epithelium and the lamina propria for the diagnostic categories.

The means of computer units of detected area for the diagnostic categories for the different phenotypes examined are illustrated in Tables 73-75.

a) Evaluation of Different Phenotypes

Comparison of the mean values seen for T1, T4, T8 and B1 phoenotypes and for T8 phenotypes identified in the epithelium is illustrated in Tables 72 and 73. The mean value in lichen planus for T1 is 33408, thus exceeding the values for ILK (30058), INT (23420) and NILK (16893). The purpose of this comparison was to identify quantifiable differences in infiltrate between the combined leukoplakia categories. It is noteworthy that T4 in LP, INT and ILK were between 10858 and 13310. In comparison, the value in NILK was 6711 which represents an

approximate reduction of 50% of this phenotype (Table 72). Also the FSK category had a similar level of T4 phenotype to the LP, INT and ILK categories. Low values (5800 and 7389) were also recorded for DLE and CL categories for the T4 phenotype. The T8 value in the INT category exceeded that of the T1 value (27850 and 23420 respectively). It is not clear what contributed to this value, however the mean values for the cases were diverse, as illustrated by the standard error of the mean (Table 72).

In the FSK category, the B1 phenotype evaluation had the lowest value (572) of all categories (Table 73). The B1 phenotype was higher in the ILK than LP categories (3119 and 2463 respectively). In leukoplakia the INT category had the lowest B1 phenotype (1532) while ILK had double at 3119. The identification of T8 phenotypes in the epithelium showed no specific trend. The LP, INT, NILK and FSK categories showed similar values for Epithelial T8 phenotype (1988, 1705, 2040 and 2182 respectively). ILK had a very much reduced quantity of this infiltrate, approximately 25% less than the categories previously mentioned.

Table 71

Patients Contributing to the Immunocytochemical Study of Oral Keratoses

		Male	Female	Total
IK				
	LP	7	6	13
	INT	2	1	3
	ILK	2	3	5
	DLE	-	1	1
	CL	2	-	2
NIK				
	NILK	2	2	4
	FSK	1	1	2
	TOTAL	15	14	30

FIGURE 20. Immunocytochemical Staining for T8 for Oral Keratosis.

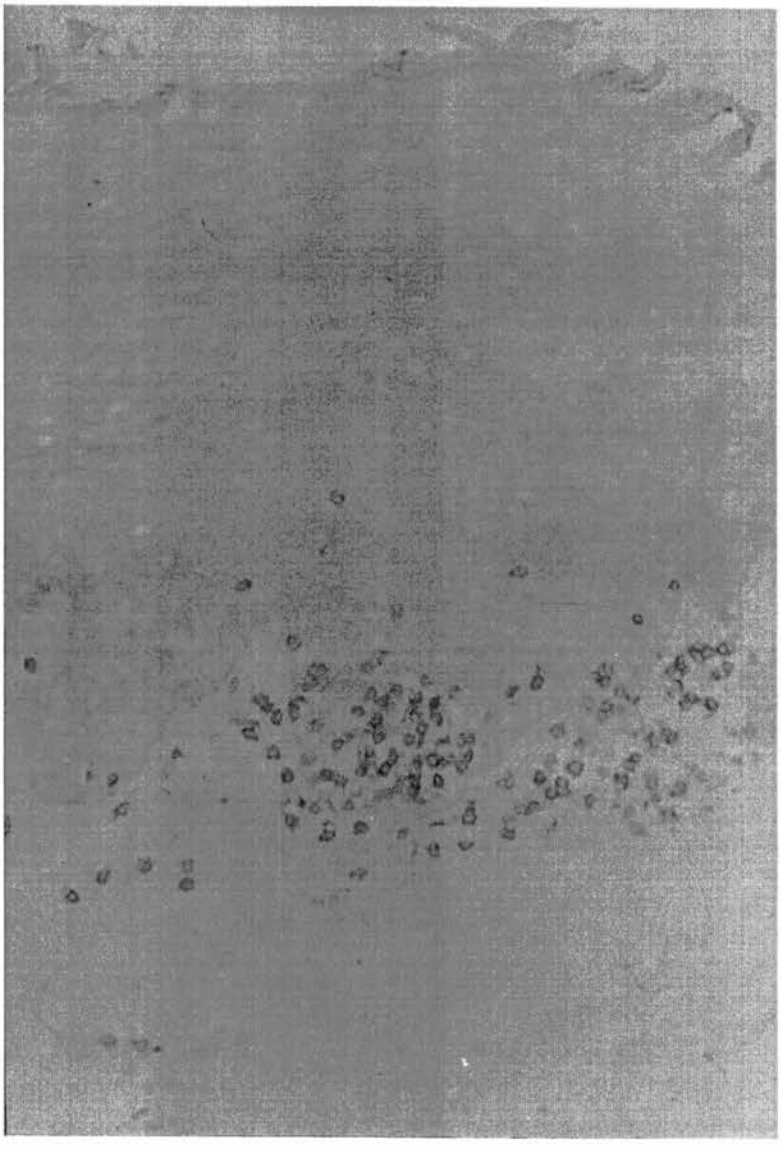


Table 72

Mean Total Detected Area for the Diagnostic Categories and the Idiotypes Investigated

	No.	T1	SEM*	T4	SEM*	T8	SEM*
IK							
LP	13	33408#	6813	13310	2812	25933	4518
INT	3	23420	5591	10858	4113	27850	10523
ILK	5	30058	3525	11718	3905	18260	4257
DLE	1	14120	-	5800	-	10040	-
CL	2	25427	5281	7389	1936	11983	4483
NIK							
NILK	4	16893	2768	6711	1791	14073	1590
FSK	2	21975	8442	12435	5576	17406	12706
OK							
TOTAL	30	29992	4348	11130	1538	21095	2561

* SEM - Standard Error of the Mean

Computer units of mean detected area.

Table 73

Mean Total Detected Area for the Diagnostic Categories and the Idiotypes Investigated

		No.	B1	SEM*	EpT8**	SEM*	Cont#	SEM*
IK								
	LP	13	2463##	638	1988	424	70	20
	INT	3	1532	629	1705	595	59	10
	ILK	5	3119	1411	516	31	137	66
	DLE	1	1248	-	7259	-	109	-
	CL	2	1893	1356	3325	2480	41	6
NIK								
	NILK	4	2620	1505	2040	782	90	21
	FSK	2	572	52	2182	1217	90	47
	OK							
	TOTAL	30	2296	415	2092	363	84	14

* SEM - Standard Error of the Mean

** Ep T8 describes the T8 phenotypes detected in the epithelium of the section

Cont - Control Sections

Computer units of mean detected area.

A broad disease comparison between lichen planus and the combined disease categories of leukoplakia was possible (Table 75). For the phenotypes T1, T4, T8 and Epithelial T8, a ratio between 1.5 and 1.3 to 1 was seen in each case, except for B1 where a ratio of 1:1 was present.

The values for the phenotypes T1, T4, T8, B1 and T8 Epith for LP, ILK, INT and NILK are shown (Table 76). NILK had a value approximately half that seen in LP for the phenotypes T1 and T4. The ILK and INT categories showed intermediate values between LP and NILK for these phenotypes. The INT category displayed the highest value for the T8 phenotype (27830) and NILK the lowest (14073). The B1 phenotype was similar in LP and NILK (2463 and 2620 respectively). INT had half the values of B1 compared to ILK (1532 and 3119 respectivley). ILK showed the lowest value for Epithelial T8 phenotype (516) and the other categories had similar values. The low T4 value in NILK, the high T8 value in INT and the low Epithelial T8 value in ILK were not found to be relevant diagnostic indicators for these categories.

Table 74

Comparison Between Lichen Planus and Leukoplakia

	LP	Leukoplakia#	Ratio
T1	33408*	21915	1.5 to 1
T4	13310	10113	1.3 to 1
T8	25933	19881	1.3 to 1
B1	2463	2556	1.0 to 1
Ep T8	1988	1385	1.4 to 1

* Mean values of detected areas.

Leukoplakia = categories ILK + INT + NILK

Table 75

Comparison Between Lichen Planus and the Respective Categories of Leukoplakia (ILK, INT, NILK)

	Diagnostic Category			
Phenotype	LP	ILK	INT	NILK
T1	33408*	30058	23420	16893
T4	13310	11718	10858	6711
T8	25933	18260	27850	14073
B1	2463	3119	1532	2620
Ep T8	1988	516	1705	2040

* Mean values of detected areas.

b) Ratios for T4/T8 Phenotypes

Ratios for T4/T8 phenotypes are illustrated in Table 77. The highest value of 0.71 for the T4/T8 ratio was seen in the FSK category. The lowest ratio was 0.39 for the INT category. In descending order ILK 0.64, CL 0.62, DLE 0.58, LP 0.51 and NILK 0.48 values were obtained. It was unclear why the FSK category showed the highest ratio and INT the lowest for this parameter. The values for T4 for categories INT and FSK were 10858 and 12435 respectively. Accepting the large variation in these values in the individual specimens, as suggested by the standard error of the mean, the relatively high level of T8 in the INT category was responsible for the T4/T8 ratio of 0.39. The NILK category, with a low value of 6711 for the T4 phenotype compared to the approximate value of 12000 for LP, INT, ILK and FSK, would suggest that relative reductions of T4 and T8 phenotypes was an important consideration for the NILK category.

Table 76

T4/T8 Ratio for the Diagnostic Categories

Category	T4/T8 Ratio
INT	0.39
NILK	0.48
LP	0.51
DLE	0.58
CL	0.62
ILK	0.64
FSK	0.71

CHAPTER 9

CELLULAR IMMUNOLOGY RESULTS

Three major aspects of in vitro cellular immunology of peripheral blood lymphocytes were investigated for oral keratoses; viz Lymphocyte transformation, T-cell mediated supression and Macrophage supression. In addition, an initial assessment of lesional cells was attempted using simple extraction techniques to obtain immunocytes from the keratotic lesions. Materials and methods used for these studies are given in chapter 3 and the controls were obtained within the dental hospital from age, sex and tobacco use matched adults without disease.

a) Lymphocyte Transformation

The mean lymphocyte transformation values obtained for patients and controls are illustrated in Table 77. Both patient and control lymphocytes obtained from peripheral blood showed stimulation with mitogens PHA, PWM and ConA but there were no significant differences. A difference between patients and controls for unstimulated lymphocytes ($p<0.013$) was found and could be of biological significance. These data may suggest greater spontaneous transformation of patient lymphocytes over those of controls.

Table 77

Lymphocyte Transformation in Patients and Controls According to Diagnostic Categories of Oral Keratoses (Mean Values)*

Diagnostic Category	Cells	O	No.	PHA	No.	PWM	No.	ConA	No.
LP	P	179	8	7039	8	625	5	3516	8
	C	142		5746		504		4106	
DLE	P	115	2	3628	2	369	2	733	2
	C	60		6146		417		1289	
SCC	P	249	3	5343	3	742	4	3456	4
	C	139		5466		631		3828	
INT	P	96	3	5008	3	610	3	4332	3
	C	73		5323		671		5427	
ILK	P	111	3	3376	4	710	3	567	2
	C	71		5067		596		2041	
NILK	P	186	4	5618	3	2553	4	562	2
	C	132		8156		1821		955	
OK##	P	164**	23	5430	24	1000	21	2794	21
TOTAL	C	114**		5868		808		3477	

S.D. omitted for clarity as data are not statistically significant

* Counts per minute (cpm).

** $p < 0.013$.

No. Number of pairs in the paired t-test.

Oral Keratoses - total contributing to the paired tests.

b) T-Cell Mediated Suppression

T-cell suppression in co-culture lymphocyte transformation tests did not provide evidence of raised or lowered T-cell suppression in patients or controls (Table 78). Similar mean values in co-cultures for T-cell mitogens PHA and ConA suggests that no significant differences in the ability of T-cells to respond to such mitogens exist between control and patient populations of peripheral blood lymphocytes. Results in later tables (Tables 80 and 81) confirm that T-cell mediated suppression was not significantly raised in patients or controls even when the influence of macrophage suppression was eliminated.

The production of immunoglobulins from co-cultures of T and B-cells was assessed using the ELISA technique described in chapter 4. The evaluation of the immunoglobulins G, A and M, as a product of T-cell suppression, was not possible due to insurmountable problems encountered with the computer software.

c) Macrophage Mediated Suppression

The contribution of macrophage suppression in lymphocyte transformation was examined by eliminating the effect of macrophages using Indomethacin and Vitamin E (Table 79). The effect of eliminating macrophage suppression generally showed enhancement of the lymphocyte transformation. However in the LP category, the spontaneous Lymphocyte transformation was significantly higher ($p<0.034$) if macrophage suppression was not eliminated, suggesting

that increased spontaneous lymphocyte transformation is present in lichen planus. Although similar differences between spontaneous lymphocyte transformation values, with and without macrophage suppression were obtained in the DLE and SCC categories, too few patients in these categories prevented statistical significance.

Table 78

Co-Culture Lymphocyte Transformation for Oral Keratoses

Diagnostic Category	Cells	O	No.	PHA	No.	PWM	No.	ConA	No.
OK	P	143*	19	7096	20	574	20	3302	17
	P/C	143		6016		486		3784	
OK	P/C	649	20	4846	19	501	19	3784	17
	C	100		5268		572		4080	

No. Number of pairs in the paired t-test.

* Mean value counts per minute (cpm).

Table 79

Macrophage Suppression of Lymphocyte Transformation for the Diagnostic Categories of Oral Keratoses (Expressed as Mean counts per minute)

Diagnostic Category	Cells	O	No.	PHA	No.	PWM	No.	ConA	No.
LP	P-#	196*	7	10998	9	528	8	3516	8
	P+##	110*		12876		581		5709	
DLE	P-	115	2	3628	2	369	2	733	2
	P+	56		5436		267		588	
SCC	P-	334	2	5343	4	742	4	3456	4
	P+	94		5964		907		4465	
INT	P-	96	3	5008	3	610	3	4332	3
	P+	306		5925		735		5155	
ILK	P-	93	4	3376	4	710	3	567	2
	P+	85		5914		1556		619	
NILK	P-	150	2	1455	1	715	2	372	1
	P+	149		1570		419		433	
OK	P-	159	24	6919	25	921	24	3692	22
TOTAL	P+	143	22	8198	24	746	24	3939	21

No. Number of pairs in the paired t-test.

Patient cells.

Patient cells: macrophage suppression eliminated with Indomethacin and Vitamin E.

* $p < 0.034$.

The effect of eliminating macrophage suppression was further assessed using co-culture tests of patient and control lymphocytes (Table 80). These data suggest that no significant differences exist between the patient and control peripheral blood lymphocytes. Furthermore, comparison of Tables 78 and 80 would indicate maintainance of lymphocyte transformation values for both patient and controls, regardless of the macrophage function.

Macrophage suppression was assessed in autologous patient and control cultures for lymphocyte transformation (Table 80). Again, as for Tables 78 and 80, enhancement of lymphocyte transformation was seen when the effect of macrophage suppression was eliminated using Indomethacin and Vitamin E. Unstimulated patient and control lymphocytes in co-culture underwent enhancement, consistent with the allogeneic effect of non-specific activation of B-cells by factors from T-cells. This effect was also seen in mixed co-culture tests for spontaneous lymphocyte transformation in Tables 78 and 80. A similar increase in lymphocyte transformation between patient, control and co-culture tests for PHA, PWM and ConA was seen. The percentage increase ranges between 10 and 20%.

Table 80

Co-Culture Lymphocyte Transformation for Patients and Control for Macrophage Suppression* in Oral Keratoses

Diagnostic Category	Cells	O	No.	PHA	No.	PWM	No.	ConA	No.
OK	P	144**	18	7658	19	640	19	3227	18
	P/C	840		6868		596		3077	
	P/C	843	18	5830	18	641	16	3077	18
	C	132		5466		634		3240	

No. Number of pairs in the paired t-test.

* Macrophage suppression eliminated by Indomethacin and Vitamin E.

** Mean value counts per minute (cpm).

Table 81

Macrophage Suppression for Autologous Patient, Control and Co-Culture for Lymphocyte Transformation

Diagnostic

Category	Cells	O	No.	PHA	No.	PWM	No.	ConA	No.
OK	P -	161*	20	6852	23	605	22	2896	20
	P +	131		8418		751		4092	
P value		NS		<0.014		NS		NS	
OK	C -	116	20	5236	23	551	19	2787	19
	C +	143		6016		646		3032	
P value		NS		NS		<0.038		NS	
OK	PC -	674	19	5885	17	502	19	2908	17
	PC +	809		6892		596		3202	
P value		NS		<0.022		NS		NS	

No. Number of pairs in the paired t-test.

NS Not significant.

\- Patient and control cells

\+ Patient and control cells with Macrophage suppression eliminated by Indomethacin and Vitamin E.

* Mean value counts per minute (cpm).

d) Functional Tests of Lesional Lymphocytes

The lesional lymphocytes from oral keratoses were only minimally responsive to mitogenic stimulation (Table 82). The oral keratoses tested, included nine patients of whom three were LP, two ILK, three SCC and one patient belonged to the NILK category. The numbers of patients indicated in Tables 82-86 varied depending upon the pairs contributing to the paired t-tests.

There was no difference in spontaneous lymphocyte transformation between peripheral blood and lesional cells. Stimulated lymphocytes transformation was always higher for peripheral blood than for lesional cellsbut the differences were only significant for PHA and ConA stimulated cultures ($p<0.01$ and $p<0.05$ respectively).

Lesional lymphocytes in each of the diagnostic categories examined (LP, ILK, SCC and NILK) were hypo-responsive to mitogenic stimulation (Table 83). Furthermore, lesional lymphocyte transformation was not suppressed by macrophages in the categories LP, ILK and SCC (Tables 84-86).

Table 82

Lymphocyte Transformation for Peripheral Blood and Lesional Lymphocytes in Oral Keratoses (expressed in mean value counts per minute)

	O	No.	PHA	No.	PWM	No.	ConA	No.
PB*	108	7	6353	8	659	6	3359	7
SEM	26.2		1890.5		188.0		1409.7	
Lesional	105	7	156	8	131	6	536	7
SEM	43.2		33.3		43.8		387.4	
Significance	NS		#		NS		##	

No. Number of pairs in the paired t-test.

* Peripheral blood lymphocytes.

SEM Standard error of the mean.

# $p < 0.01$.

$p < 0.05$.

Table 83

Lesional Lymphocyte Transformation for Four Diagnostic Categories of Oral Keratoses

	O	PHA	PWM	ConA
LP	34 (2)*	205 (1)	39 (2)	70 (2)
SEM	14	-	5	31
ILK	49 (2)	71 (2)	46 (2)	-
SEM	19	25	12	-
SCC	223 (2)	212 (2)	213 (2)	239 (2)
SEM	85	94	82	88
NILK	229 (1)	303 (1)	224 (1)	264 (1)
SEM	-	-	-	-

SEM Standard error of the mean.

* Number of pairs in the paired t-test.

Table 84

Lymphocyte Transformation for Lesional Cells with and without Macrophage Suppression in Lichen Planus (expressed in mean value counts per minute)

Cells	O	No.	PHA	No.	PWM	No.	ConA	No.
Lesional-	33.6	2	204.6	1	39.3	2	69.6	2
SEM	14.6		-		5.3		31.6	
Lesional+	44.0	2	45.6	1	61.5	2	92.0	
SEM	6.0		-		6.1		23.3	
Significance	NS		NS		#		NS	

No. Number of pairs in the paired t-test.

\- Patient cells

\+ Patient cells with macrophage suppression eliminated by Indomethacin and Vitamin E.

\# $p < 0.02$.

Table 85

Lymphocyte Transformation for Lesional Cells with and without Macrophage Suppression in Infiltrated Leukoplakia (ILK)

(expressed in mean value counts per minute)

Cells	O	No.	PHA	No.	PWM	No.	ConA	No.
Lesional-	49.0	2	71.0	1	46.3	1	-	-
SEM	19.3		25.3		12.0		-	
Lesional+	72.8	2	94.0	1	59.0	1	-	-
SEM	45.1		68.3		28.0		-	
Significance	NS		NS		NS			

No. Number of pairs in the paired t-test.

\- Patient cells

\+ Patient cells with macrophage suppression eliminated by Indomethacin and Vitamin E.

Table 86

Lymphocyte Transformation for Lesional Cells with and without Macrophage Suppression in Squamous Cell Carcinoma (SCC)

(expressed in mean value counts per minute)

Cells	O	No.	PHA	No.	PWM	No.	ConA	No.
Lesional-	223.3	2	212.0	2	213.3	2	239.3	2
SEM	84.6		94.3		82.0		88.0	
Lesional+	145.5	2	145.0	2	190.3	2	164.8	2
SEM	30.8		31.0		92.6		63.8	
Significance	NS		NS		NS		NS	

No. Number of pairs in the paired t-test

\- Patient cells

\+ Patient cells with macrophage suppression eliminated by Indomethacin and Vitamin E.

Co-cultures were set up to examine the influence of lesional lymphocytes on peripheral blood lymphocytes (Table 87).

The suppressor capacity of the lesional lymphocytes was assessed by comparing the separate transformation values for cultures of lesional and peripheral blood lymphocytes, with the co-cultures of these cells. The mean transformation value for the peripheral blood lymphocytes and the lesional cells was used as a reference point and was called the expected value. The value obtained for the co-culture of peripheral blood lymphocytes and the lesional cells was called the actual value. If the actual value was greater than the expected value, the combination of the cells in the co-culture was suggestive of immunological enhancement. Similarily, if the actual value of the co-culture was lower than the expected mean value, then immunological suppression was interpreted. The actual value could be calculated as a percentage of the expected value and these data are shown in Table 88.

It is seen that immunological suppression is the predominant response of the co-cultures with the combination of oral keratoses (OK) producing the greatest value of 632% with ConA stimulation. It is further seen that the SCC category produced a higher pecentage of suppression with PHA stimulation than other categories, suggesting that the lesional immunocytes exert a strong suppressive influence on the peripheral blood lymphocytes in co-culture. It may be relevant that what is seen as minimal lesional immunological enhancement in ILK with PHA, contrasts with the strong immunological suppression seen in the SCC category with the same mitogenic influence.

Table 87

Co-Cultures Between Lesional and Peripheral Blood Lymphocytes in Oral Keratosis and its Diagnostic Categories (expressed in mean value counts per minute)

Diagnostic Category	Cells	O	No.	PHA	No.	PWM	No.	ConA	No.
LP	L	34	2	155	2	39	2	995	3
	L/PB	-	-	-	-	-	-	-	-
	PB	81	2	11705	2	723	2	6849	3
ILK	L	46	3	62	3	58	1	23	1
	L/PB	72	2	2908	3	340	2	206	2
	PB	98	3	4017	3	1460	1	836	1
SCC	L	308	1	202	3	213	2	239	2
	L/PB	199	1	783	2	266	2	339	2
	PB	86	1	6754	3	370	2	882	2
NILK	L	229	1	303	1	224	1	264	1
	L/PB	209	1	798	1	189	1	244	1
	PB	221	1	1455	1	310	1	373	1
OK	L	106	7	156	9	131	6	536	7
	L/PB	-	-	1847	7	280	5	266	5
	PB	109	7	6353	9	659	6	3359	7

No. Number of pairs in the paired t-test.

L Lesional lymphocytes.

L/PB Co-culture of lesional and peripheral blood lymphocytes.

PB Peripheral blood lymphocytes.

Table 88

T-Cell Suppression of Lymphocyte Transformation by Lesional Lymphocytes in Oral Keratoses

Category	PHA	PWM	ConA
LP	-	-	-
ILK	(30%)*	123%	109%
SCC	344%	10%	66%
NILK	10%	(14%)	31%
Combined Oral Keratoses (OK)	76%	41%	632%

Values are approximate as paired t-tests for L/PB co-cultures were fewer than autologous cell cultures (see Table 87).

The values in brackets indicate immunological enhancement in the co-cultures. Refer to text for explanation.

* %suppression(enhancement) = actual counts per minute(cpm) x 100
expected counts per minute(cpm)

where actual cpm = cpm of co-culture and

expected cpm = mean cpm of patient cells + cpm of lesional cells

CHAPTER 10

DISCUSSION

Oral keratoses present clinically and pathologically with a broad range of features. The literature review shows the importance of obtaining a specific diagnosis of an individual keratosis whenever possible, as diagnosis generally determines management and the risk of malignant change varies between different keratoses. Diagnosis remains partly subjective and no strict objective criteria have emerged which can determine diagnosis or confidently predict the development of malignant change. The diagnosis of both leukoplakia and lichen planus is often straight forward when both clinical and histological examinations have been completed. The histological features of lichen planus are not as variable as those of leukoplakia and the pathologist is limited by strict histological criteria when diagnosing lichen planus. Although a review of the literature suggests that distinction between these two conditions is generally clear cut, the present study shows that some patients present with lesions which are not easy to diagnose. This is particularly difficult when a patient presents with features of both conditions or a change is seen within a specific lesion over the course of time.

During the course of the work involved in the present study, it became apparent that it might be helpful to distinguish those lesions which had an inflammatory infiltrate (whether in a particular pattern as in lichen planus or in a more heterogeneous pattern as in leukoplakia) from those lesions in which an inflammatory infiltrate was absent.

A different classification of keratoses from that generally in current use was adopted, but the classification could easily be compared with those used in previous studies. While it may be preferable in an ideal study to have similar numbers of patients in all diagnostic categories for comparison, a prospective study over three years of patients with keratoses presenting in a hospital did not allow such and opportunity.

Some of the findings in the present study were consistent with those previously reported. For example the higher incidence of lichen planus in females contrasting with that of males in leukoplakia, the high frequency of smoking in leukoplakia and the combination of tobacco and alcohol use in squamous cell carcinoma.

No obvious differences could be detected in the haematological and biochemical results from patients in the different diagnostic categories which could be of value in establishing a diagnosis. A comparable proportion of oral keratosis (OK) patients (14.4%) were found to have a similar range of deficiencies compared to the patients reported in the study by Wray et al (1975) where 130 RAS patients produced 23 patients (17.7%) with deficiency (241).

	OK*	RAS*
Iron	12	15
Folate	3	7
B12	9	5
Total deficient	23 (14.4%)	23 (17.7%)
Study Total	159	130

The diagnostic categories of lichen planus and non-infiltrated leukoplakia had a higher proportion of patients presenting deficiencies, 22.5% and 19.2% respectively, than the RAS group (241) suggesting haematological screening may be of value for these categories.

Nutritional deficiencies were found in a few patients with oral keratoses, but whether these deficiencies are the cause rather than the result of the disease has not been established, nor has it been determined whether the deficiency is dietary or not. As part of a larger study of patients with oral mucosal disease, a few patients with oral keratoses were investigated for gut permeability. No abnormalities were found in the non-infiltrated leukoplakia group while more than half of the patients with infiltrated leukoplakia were determined to have abnormal permeability. The significance of this finding is unclear at present.

The intermediate keratosis category was introduced in the classification because a group of lesions did not have the criteria either for inclusion into lichen planus and the infiltrated keratoses, or for inclusion into the non-infiltrated keratoses. These lesions were termed intermediate because of the likelihood that they represented an intermediate state of disease. It was surprising therefore that the intermediate category had significantly lower serum immunoglobulin levels than the lichen planus, infiltrated leukoplakia and non-infiltrated leukoplakia categories suggesting that systemic immunoglobulin changes may be related to the intermediate categories only. Dermatological patch testing suggested that patients with

infiltrated lesions (particularly infiltrated leukoplakia) had a greater sensitivity to nickel and mercury than those with non infiltrated leukoplakia, but this was not matched by differences in the lymphocyte transformation. A relationship between mercury and nickel sensitivity and the presence of amlagam restorations adjacent to lesions of lichen planus could not be established.

In the present study, possible lichenoid drug reactions were not distinguished within the lichen planus category and it was expected that this category might show an increased use of drugs when compared with the other categories where no relationships with drug histories have been shown. It was found however that there was no obvious difference in the frequency of patients taking drugs between any of the diagnostic categories. In addition, although the overall incidence of drug taking was higher in the non-erosive than in the erosive lichen planus patients, there was a distinct increase in the number of patients using non-steroidal anti-inflammatory drugs in the patients with erosive lesions. The use of non-steroidal anti-inflammatory drugs by patients with erosive lichen planus compares closely to those values for patients with erosive lichen planus reported by Potts et al (1987)(395).

The associated presence of autoimmunity was monitored by measuring ANF levels which were found to be raised in patients in all disease categories which included more than three patients. It was concluded that the presence of ANF did not help in the diagnosis of the known autoimmune disease discoid lupus erythematosus when presenting as an oral keratosis.

The literature review details the characteristic histological features of keratoses such as lichen planus and discoid lupus erythematosus, the diagnosis of which may be entirely based on histology. This contrasts with other keratoses such as frictional keratosis and leukoplakia where the histological features alone are insufficient for a definitive diagnosis. There are however some patients with lesions which histologically do not conform to the strict criteria for the diagnosis of lichen planus but which clinically do conform. For management, it is preferable to regard them as having infiltrated keratoses and if the clinical criteria are sufficiently pronounced they should be managed as lichen planus. One of the purposes of the histological study was to attempt to assess which criteria of lichen planus were most helpful in diagnosis and which criteria were less helpful in that they are also commonly present in other keratoses.

Keratinisation may be ortho or para and, if the variation in thickness of the keratin is ignored, Table 64b shows that parakeratin is the "dominant" type in lichen planus and frictional and smoking keratosis with intermediate and non-infiltrated leukoplakia showing orthokeratin as the "dominant" type. In the other keratoses the frequencies were more balanced. The presence or absence of a granular layer was not found to be helpful. Epithelial thickness was not found to be helpful in diagnosis, although atrophy featured more commonly in the infiltrated than in the non-infiltrated keratoses. Liquefactive degeneration was rarely seen in non-infiltrated leukoplakia but this was not surprising in that it is generally regarded as being associated with inflammatory oedema. For that reason it was commonly

seen in infiltrated keratoses. A thickened basement membrane, while not commonly seen, was almost entirely a feature of lichen planus and discoid lupus erythematosus. The frequency of apoptosis was similar to that of liquefactive degeneration, reflecting increased cell turnover. The most consistent histological feature of lichen planus was the almost universal presence of a juxta-epithelial, band-like infiltrate with no associated infiltrates in the deeper connective tissue. In lichen planus this infiltrate was almost exclusively composed of lymphocytes and macrophages. This contrasts with other types of infiltrated keratoses where the juxta-epithelial infiltrate was sometimes band-like, with a tendency for infiltrates in the deeper connective tissue also, the infiltrates having a much more variable composition with plasma cells and polymorphs also present (Tables 67 and 68). Dysplasia was detected in approximately 50% of infiltrated leukoplakia biopsies and in 30% of lichen planus biopsies. The presence of dysplasia in keratotic lesions is thought to be of value in predicting malignant change, but the presence of dysplasia in lichen planus appears to be related to the inflammatory infiltrate and not a predictor of malignant change. As explained earlier, the intermediate category was introduced for those cases which, for one reason or another, did not conform with the criteria required for diagnosis of the other categories. When the histological features of patients with intermediate diagnoses were assessed, the results suggested that intermediate was a valid category linking non-infiltrated leukoplakia with infiltrated leukoplakia in that the type of keratinisation and the nature of the infiltrates resembled infiltrated leukoplakia while the basement membrane and frequency of dysplasia resembled non-infiltrated leukoplakia.

The 30 sections stained for mononuclear cells provided a comparison of immunological infiltrates between oral keratoses. Previous studies by Ishii (1987), Migliorati et al (1986) and Takeuchi et al (1988) have been restricted to lichen planus or leukoplakia (349,360,397). Antigen presentation by monocytes and Langherhans cells is suggested in lichen planus and altered T4/T8 ratios are associated with epithelial dysplasia (349,360).

In the present study, B1 phenotype lymphocytes were found in the connective tissue infiltrate both in lichen planus and a combined leukoplakia group consisting of infiltrated leukoplakia, intermediate and non infiltrated leukoplakia. This monoclonal antibody detects both B cells and plasma cells. Plasma cells are very infrequently seen histologically in lichen planus and therefore B1 staining in lichen planus suggests that B cells may be resident in the lichen planus infiltrate but are not maturing into plasma cells. This is consistent with the reported predominate of cell mediated activity related to T cells in lichen planus.

Quantifiable differences in the non-B cell population of lymphocytes were observed between diagnostic categories (Tables 72-74). The non-infiltrated leukoplakia category showed lower values of connective tissue infiltrate for T1, T4 and T8 phenotypes than the frictional and smoking keratosis category. This comparison may suggest that an immunological hyporesponsive state exists for the non- infiltrated leukoplakia category. Furthermore, ten patients with leukoplakia of the floor of mouth were identified within the non-infiltrated leukoplakia category (Table 27xv).

The assessment of T8 infiltrate in the epithelium was seen to be similar for lichen planus, intermediate leukoplakia, non-infiltrated leukoplakia and the frictional and smoking keratosis category, with much higher values in discoid lupus erythematosus and candidal leukoplakia categories and lower values in the infiltrated leukoplakia category (Table 73). The T4/T8 ratio reflects the numerical relationship of helper to suppressor lymphocytes in a lesion. As the proportion of T4 lymphocytes in the intermediate category is similar to that in the lichen planus and infiltrated leukoplakia categories, the increase of T8 phenotype is largely responsible for the low T4/T8 ratio of 0.39 in the intermediate category suggesting that more suppressor/cytotoxic activity is involved in intermediate leukoplakia compared to lichen planus or infiltrated leukoplakia (table 76).

The cellular immunological tests were unhelpful in providing diagnostic indicators for the categories of oral keratoses. The data were presented in a basic form of mean counts per minute and although calculation of the stimulation index from the data was possible, this was not thought appropriate (388). The biological significance of the difference between spontaneous patient and control lymphocyte transformation ($p<0.013$) is unclear (Table 77). Recent evidence that soluble serum interleukin 2 receptor is associated with disease activity of atopic eczema may, however, be useful in explaining this difference in lymphocyte transformation (398). The reduced lymphocyte transformation in peripheral blood lymphocytes in lichen planus with elimination of macrophage suppression is not of biological significance (Table 79). These data suggest that the basic lymphocyte proliferative responses remain intact for all categories of oral

keratoses and that macrophage suppression does not account for a significant effect on the proliferation of peripheral blood lymphoctyes under T and B cell mitogen stimulation. It is recognised that lymphocyte transformation provides a 'rough' estimation of the cellular immune functions.

Data illustrated in Table 81 do, however, suggest that in both autologous and mixed patient and control cultures, significantly greater effects of macrophage suppression may indeed be a feature of patient lymphocytes when compared to control lymphocytes ($p<0.014$ and $p<0.022$ respectively). Again, activation of macrophages via soluble cytokines or interleukin 2 can be speculated (398).

In an attempt to identify cellular immune information on the inflammatory infiltrates of oral keratoses, biopsies were used to extract available immunocytes. The technique was hindered by the limited amount of infiltrate and size of biopsy possible. Where lesional cells were extracted and separated from lesional tissue debris by using the ficoll-hypaque separation technique, the cells appeared unresponsive to mitogenic stimulation (Tables 83-86). This aspect was further investigated by utilising co-culture experiments and thereby obtaining an indication of the suppressor effect that the lesional cells retain. It was also anticipated that, following the harsh extraction process, the cellular capacities may be reduced. Furthermore, histopathological sections of the tissue processed for lesional immunocytes showed that not all lymphocytes had been extracted. The data therefore should be regarded with these observations in mind.

Large amounts of tissue were obtained from squamous cell carcinomas and the lesional immunocytes extracted appear to have a strong suppressive capacity (Table 88). The values for oral keratoses collectively appear strongly influenced by the squamous cell carcinoma category response. It would therefore appear that strong suppressive capacities are present in the lesional cells extracted from squamous cell carcinoma. This initial study will require further data to confirm these results.

Summarising the main aspects of this thesis, a number of observations can be made. Some of these observations confirm what was already generally accepted, while others have not been described previously but require further investigation for confirmation. A number of the observations answer the questions listed at the end of the Literature Review (page 97) outlining the objective of the present study. The following observations confirm previous findings which are generally accepted:-

1. Oral keratoses present a broad spectrum of clinical and pathological features.
2. The clinical presentations of the different categories of oral keratoses were not specific to each category.
3. Tobacco and alcohol are positively associated with categories infiltrated leukoplakia, discoid lupus erythematosus, candidal leukoplakia, squamous cell carcinoma and non infiltrated leukoplakia. Patients with lichen planus smoke less tobacco and drink significantly less alcohol than certain other categories including infiltrated leukoplakia, non infiltrated

leukoplakia and squamous cell carcinoma.

4. Quantitative differences are detectable in the immunocyte infiltrates in the different diagnostic categories of oral keratosis.

The following observations have either not been reported previously or provide further evidence to support results which are not generally accepted:-

1. Haematological deficiencies were not related to particular types of oral keratoses with the exception of lichen planus and non-infiltrated leukoplakia.
2. Drugs are frequently taken by patients in all categories of oral keratoses. The use of non-steroidal anti-inflammatory drugs may be related to erosive forms of lichen planus rather than to non-erosive forms.
3. Quantitative differences in both alcohol consumption and tobacco smoking between the diagnostic categories exist
4. Abnormal intestinal permeability to sugars was found in patients with infiltrated keratoses but not in those with non-infiltrated keratoses.
5. Serum immunoglobulin levels are similar for all categories of oral keratoses except for patients in the intermediate leukoplakia category of oral keratosis whose serum immunoglobulin levels were reduced.
6. While basic cellular immunological tests have been unhelpful, as of yet, in determining major functional abnormalities in oral keratoses, the squamous cell carcinoma category presented lesional

immunocytes which had strong suppressive capacities.

The hypothesis that the cellular infiltrate, present or absent from the oral keratosis, is important in determining the malignant potential of the lesion, cannot be rejected on the evidence of the results presented. While it was not possible to relate malignant change with the cellular infiltrates preceeding the malignancy, the T4/T8 ratios (Table 76) suggest polarisation between the categories of leukoplakia. The T4/T8 ratios and the tobacco and alcohol history may be of value in the clinical management of oral lichen planus and leukoplakia.

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